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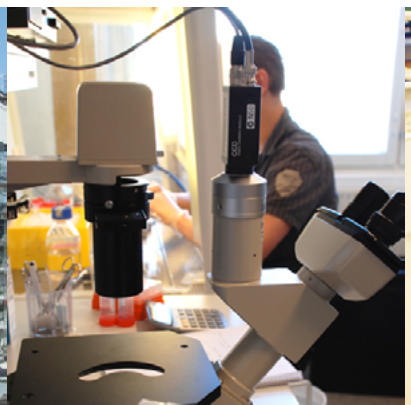
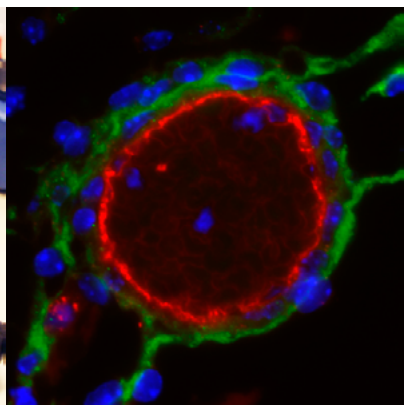
Department of Immunology, Genetics and Pathology

Annual Report 2015



Annual Report

2015

Department of Immunology, Genetics and Pathology

Uppsala University

The Rudbeck Laboratory
SE-751 85 Uppsala
Sweden

Uppsala Biomedical Centre
Box 815
751 08 Uppsala
Sweden

www.igp.uu.se

Editor: Kerstin Henriksson (kerstin.henriksson@igp.uu.se)
Photos/images: Sara Bolin, Johan Brännström, Axel Henriksson, Kerstin Henriksson, HPA, Molecular Tools, V. Tolmachev's research group

Introduction

This is the yearly report from the Department of Immunology, Genetics and Pathology (IGP). As Head of Department, I first want to thank all who are affiliated with IGP: employees, students, clinicians, associated scientists and everyone else for a fantastic year. Looking back on 2015, we clearly see a year marked by success and achievements.

An important milestone was that IGP completed the fusion with Oncology, Biomedical Radiation Sciences and Medical Radiation Physics units from the former ROS department. The process of incorporating employees and students was initiated in 2014 and continued throughout 2015. We have restructured our research programmes to better reflect the new structure of the Department, and to welcome the new groups to IGP. A festive "Fusion Event" was organized in February for all IGP to celebrate the fusion. In May, the PhD Student Council arranged a conference so that graduate students from the old and new parts of our department would get to know each other's research. The conference was held at Campus Gotland. The fusion opens new possibilities for increased contacts across many research areas and also enables fruitful interactions with the two additional affiliated clinics, Oncology and Hospital Physics. This became obvious during the IGP Day in June, where more than 150 IGP researchers got together for a day of scientific presentations, enjoying a program of oral presentations and discussions around posters.

Many principal investigators at IGP were successful in attracting prestigious grants and awards during 2015. Warm congratulations to everyone! It is impressive that three ERC grants went to IGP researchers; Lars Forsberg received a Starting Grant, Taija Mäkinen a Consolidator Grant and Ulf Landegren a Proof-of-Concept Grant. From Knut and Alice Wallenberg Foundation, a major project grant was awarded to Christer Betsholtz, Lena Claesson Welsh, Elisabetta Dejana, Taija Mäkinen and Katie Bentley. Christer Betsholtz also received a Swedish Research Council (VR) grant for Distinguished Professors. Åsa Johansson was awarded a project grant to Young Researchers from VR, and in addition, six VR project grants went to IGP group leaders. Nine grants from the Swedish Cancer Society were approved and from the Swedish Childhood Cancer Foundation IGP researchers received four project grants, two NBCNS grants and two post doc grants. Furthermore, Torsten Söderbergs stiftelse awarded Jan Dumanski a major grant. A grant was also given to the Human Protein Atlas through a new Wallenberg Centre for Protein Research, with project leader Cecilia Lindskog Bergström at IGP. Here I can only mention a selection of all grants, but each one is important, large as small, and will be put in the best of use for research at IGP. I thank everyone for their efforts in applying for external grants, which is absolutely critical for the continued growth and prosperity of the Department.

Several researchers at IGP received prizes for their scientific achievements. The Hilda and Alfred Eriksson Prize was given to Per Westermark, Taija Mäkinen received Eric K Fernströms Svenska Pris, Hannah Karlsson the Hwasser prize for best pre-clinical PhD thesis and Hadis Honarvar a Marie Curie Award for her conference contribution.

Of note regarding higher education, IGP is now a partner in an EU funded European Master Program, IMIM, together with universities in Groningen and Heidelberg, allowing Master students to rotate between the three universities. A special thanks to Programme Director Lena Åslund and IGP's Head of Education Nils-Erik Heldin, for their continuous hard work to ensure that education at IGP remains of the highest quality. Recent activities towards this goal is the creation of a student project catalogue on the IGP web, and arranging an information evening for medical students (T7) about ongoing research at IGP, to stimulate

their interest in our projects. With the new Department structure we are also happy to host the master program in Medical Nuclide Techniques.

In the evaluation of strategic research environments, projects connected with IGP received high ratings in all three evaluated areas. IGP researchers have major roles in four projects that received funding in the Strategic Research Area Initiative, launched by the Swedish Government in 2010. These are U-CAN and SciLifeLab, which are formally managed by Uppsala University, and EXODIAB and StemTherapy that are managed from Lund, but with a substantial involvement from Uppsala University. Both SciLifeLab and U-CAN received the rating *Excellent* in all evaluated areas; performance, strategy and added value for the future. EXODAB and StemTherapy also had a favourable outcome. Based on the evaluation results, the funding agencies involved in managing the grants have recommended the Swedish government that funding for strategic research projects should continue to the same extent.

During 2015, the first extension of the Rudbeck Laboratory, the R3 wing, was inaugurated with new lab and office space completed. It was, and still is, exciting but also challenging to continue the research and educational activity during the reconstruction phase.

The overriding common goal of IGP's research activities is to improve prevention, diagnostics and treatment of diseases. As Head of Department, I strive to support this endeavour to the best of my capacity. I gratefully acknowledge all who assist in these efforts: the excellent IGP administrative staff, project coordinators at the Disciplinary Domain of Medicine and Pharmacy, the Grants Office, the Legal Affairs Division, UU Innovation, and the Central University Administration.

Karin Forsberg-Nilsson
Head of Department

Contents

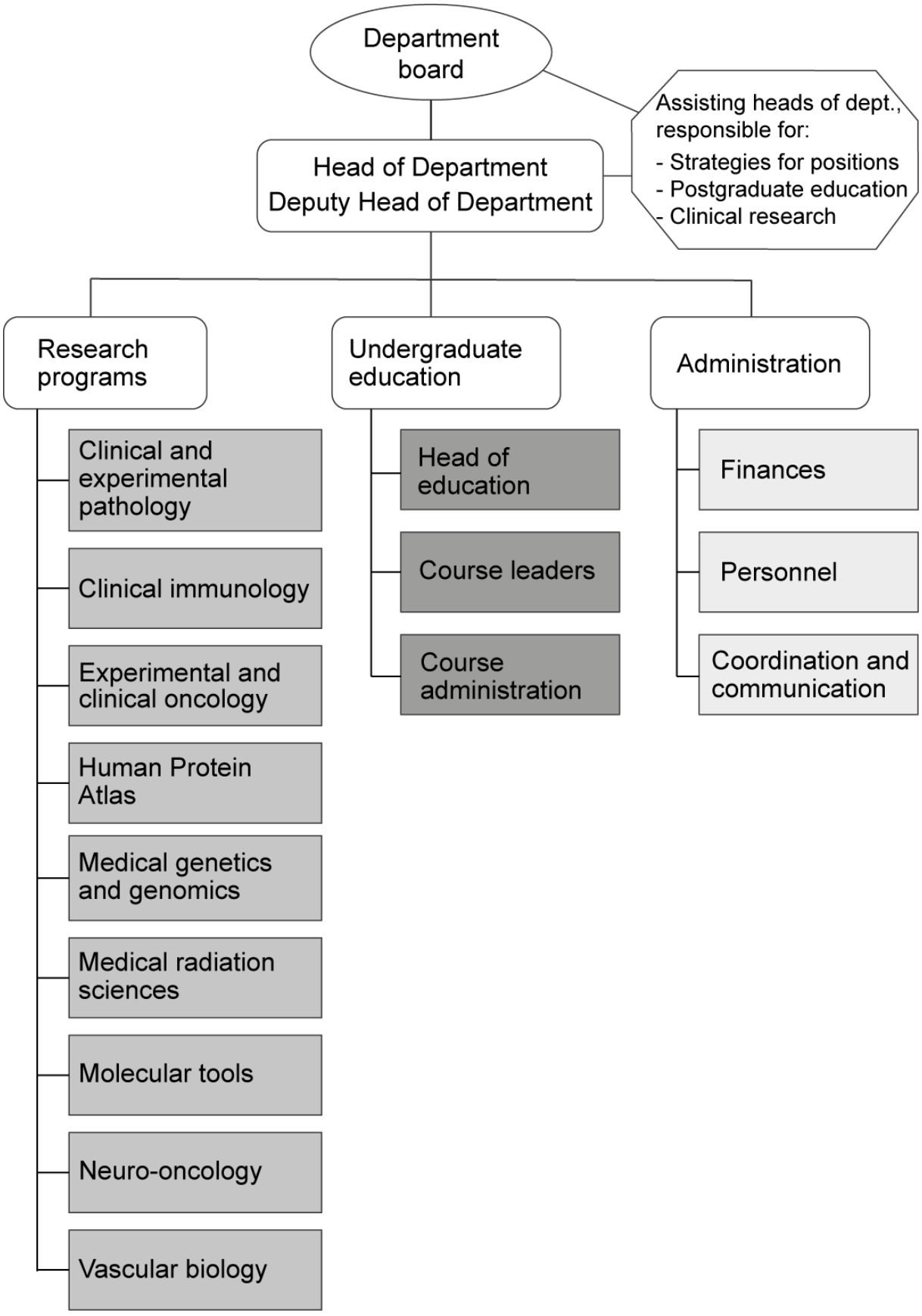
Employees during 2015

Abramenkovs Andris
Abu Taha Abdallah
Adler Jeremy
Adlerteg Tom
Ahlander Anders
Ahlstav Suzanne
Ahnesjö Anders
Alafuzoff Irina
Al-Amin Abdullah
Alemayehu Groom
Allen Marie
Almstedt Elin
Altai Mohamed
Alzrigat Mohammad
Ameur Adam
Anagandula Mahesh Kumar
Anand Manivel Vivek
Andaloussi Mäe Maarja
Andersson Karl
Andersson Magdalena
Andrae Johanna
Arngården Linda
Aronsson Maria
Asif Sana
Asp Michaela
Asplund Anna
Atienzia Párraga Alba
Atterby Christina
Backeryd Lindström Anna
Barbu Andreea
Baskaran Sathishkumar
Bentley Katie
Berggrund Malin
Berglund Maria
Bergman Julia
Bergström Tobias
Betsholtz Christer
Bhoi Sujata
Björkesten Johan
Bladin Emelie
Bodare Sofia
Boersma David
Bolin Sara
Bonnedahl Catrine
Boox Pirkko
Borgenvik Anna
Broström Ulrika
Brännström Johan
Bunikis Ignas
Bus Magdalena
Cancer Matko
Cavalli Marco
Chen Lei
Chmielniakova Jana
Chugunova Elena
Claesson-Welsh Lena
Cortese Diego
Cunha Sara
Dahl Niklas
Dalmo Erika
Danielsson Marcus
Davies Hanna
Dejana Elisabetta
Dimberg Anna
Djerf Jenny
Djureinovic Dijana
Dohlmar Ulf
Dumanski Jan
Dührkop-Sisewitsch Claudia
Ebai Tonge Brunhilda
Edqvist Per-Henrik
Edvinsson Åsa
Ek Weronica
Ekberg Elin
Elbagir Sahwa
Elfineh Lioudmila
Enblad Gunilla
Enroth Stefan
Eriksson Emma
Ericsson Maja
Essand Magnus
Etemadikhah Mitra
Falk Sörqvist Elin
Feuk Lars
Fletcher Erika
Fonnaland Karin
Forsberg Lars
Forsberg-Nilsson Karin
Forslund Marina
Fotaki Grammatiki
Frejd Fredrik
Fromell Karin
Frye Maike
Gallant Caroline
Galli Joakim
Gallini Radiosa
Gammelgård Gustav
Garousi Javad
Gedda Lars
Georganaki Maria
Gouveia Leonor
Grönlund Eric
Gu Jijuan
Gubanova Evgenia
Gudmundsson Sanna
Gupta Rajesh
Gustafsson Birgitta
Gustafsson Ida
Gustafsson Karin
Gustavsson Inger
Gyllensten Ulf
Gängel Konstantin
Halvardson Jonatan
Hamad Osama
Hansson Tony
He Liqun
Hedlund Lindberg Julia
Hedlund Marie
Heldin Johan
Heldin Nils-Erik
Hellström Ann-Charlotte
Hellström Mats
Henriksson Kerstin
Hermansson Annika
Herö Johanna
Hikmet Noraddin Feria
Hjertström Östh Inger
Holmberg Olausson Karl
Holmfeldt Linda
Honarvar Hadis
Hong Jaan
Huang Hua
Huminiecki Lukasz
Hutter Sonja
Häggqvist Susana
Höijer Ida
Ilbäck Carolina
Ingvast Sofie
Israelsson Katarina
Jeansson Marie
Jernberg Wiklund Helena
Jin Chuan
Johansson Birgitta
Johansson Patrik

Johansson Swartling Fredrik
Johansson Åsa
Jonasson Inger
Jung Bongnam
Jönsson Jennifer
Kalushkova Antonia
Kamali-Moghaddam Masood
Kampf Caroline
Karlsson Hannah
Karlsson Marie
Karlsson Torgny
Karlsson-Parra Alex
Kastemar Marianne
Katona Borbala
Kesti Dennis
Klaesson Axel
Klar Joakim
Korsgren Olle
Krona Cecilia
Kuhnemund Malte
Kundu Snehangshu
Kundu Soumi
Kuric Enida
Källström Lillemor
La Fleur Linnea
Laan Loora
Ladenvall Claes
Landegren Ulf
Larsson Chatarina
Lavina Siemsen Barbara
Leja-Jarblad Justyna
Lindahl Erik
Lindau Cecilia
Lindholm Carlström Eva
Lindskog Bergström Cecilia
Lindström Anne-Christine
Ljungström Viktor
Loganathan Krishnapriya
Loskog Angelica
Lundberg Marcus
Löf Liza
Lönn Peter
Lönnstedt Ingrid
Magnusson Christina
Magnusson Peetra
Mangsbo Sara
Mansouri Larry
Marques Souza de Oliveira
Martikainen Miika
Martikainen Minttu-Maria
Martinez-Corral Ines
Martinsson Pernilla
Mathot Lucy
Mattsson Johanna
Maturi Naga Prathyusha
Melin Malin
Milennova Yoanna
Molnar Matyas
Morin Eric
Mortensen Anja
Mosbech Mai-Britt
Mulder Sara
Muppani Naveen Reddy
Müller Malin
Mäkinen Taija
Nahar Khayrun
Nelander Sven
Nestor Marika
Niaudet Colin
Niazi Adnan
Niklasson Mia
Nilsson Berith
Nilsson Bo
Nilsson Camilla
Nilsson Ekdahl Kristina
Nilsson Mats
Nitzsche Anja
Nong Rachel
Nordling Sofia
Norlin Helene
Nygren Peter
Näsström Åsa
Oelrich Johan
Ohlin Sjöström Elisabet
Oldenburg Joppe
Olsson Cecilia
Olsson Ingmarie
Oroujeni Maryam
Ortsäter Henrik
Pacholsky Dirk
Padhan Narendra
Pan Gang
Paul-Wetterberg Gabriella
Persson Jonas
Peterson Pia
Petersson Sara
Petri Anna
Pettersson Ulf Gösta
Pontén Fredrik
Qiujin Shen
Quarfordt Pernilla
Ramachandran Mohanraj
Rasi Chiara
Rask-Andersen Mathias
Raykova Doroteya
Rendo Verónica
Roche Francis
Rosén Gabriela
Rosenquist Brandell Richard
Roy Ananya
Rung Johan
Rönnelid Johan
Sabelström Hanna
Sandberg Charlotta
Sandgren Camilla
Savov Vasil
Schenström Maria
Schmidt Linnéa
Schuster Jens
Simu Tuulikki
Sjöberg Susanne
Sjöblom Tobias
Sjöstedt Evelina
Sjösten Anna
Skog Oskar
Smeds Patrik
Smith Ross
Sohrabian Azita
Spiegelberg Diana
Spyrou Argyris
Staaf Elina
Stamatopoulos Konstantino
Staxäng Karin
Steffen Henrik
Steimer Ulla
Stenerlöw Bo
Stoimenov Ivaylo
Strand Ann-Sofi
Stratmann Svea
Sun Ren
Sundell Veronica
Sundström Anders
Sutton Lesley Ann
Söderberg Ola
Tan E-Jean
Tegehall Angelica
Tellgren-Roth Christian
Teramura Yuji
Testini Chiara
Tibbling Gunilla Birgitta

Tolmachev Vladimir
Uhrbom Lene
Ullbors Anna-Maria
Ullerås Erik
Ulvmar Maria
Wadelius Claes
Wagenius Sofie
van Hooren Luuk
Weishaupt Holger
Wenthe Jessica
Wester Kenneth
Westermark Ann
Westermark Bengt
Wicher Grzegorz
Wikner Charlotte
Villegas Navarro Fernanda
Williams Nina
Vinnere Pettersson Olga
Vuu Jimmy
Xiong Anqi
Young Emma
Yu Di
Yuan Xie
Zaghlool Ammar
Zhang Lei
Zhang Yang
Zhao Hongxing
Zhao Jin
Zieba Agata
Åslund Lena

Organisation of the Department of Immunology, Genetics and Pathology



Head of Department

Karin Forsberg Nilsson

Vice Head of Department

Ulf Landegren

Assistant Heads of Department

Claes Wadelius, postgraduate education
Bo Nilsson, clinical research
Bo Stenerlöw, recruitments

Department Board

Members during 2015

Karin Forsberg Nilsson, Head of Department
Christer Betsholtz, teacher representative
Niklas Dahl, teacher representative
Anna Dimberg, teacher representative
Elin Ekberg, representative for technical/administrative staff
Lars Feuk, teacher representative
Sarah Galien, undergraduate student representative
Marie Hedlund, representative for technical/administrative staff, deputy
Masood Kamali-Moghaddam, teacher representative, deputy
Inger Jonasson, representative for technical/administrative staff, deputy
Ulf Landegren, teacher representative, deputy
Viktor Ljungström, graduate student representative
Sven Nelander, teacher representative, deputy
Sara Nordling, graduate student representative, deputy
Ola Söderberg, teacher representative
Sawin Yousef, undergraduate student representative

Teaching organisation

Nils-Erik Heldin, head of education
Sofia Bodare, course administrator
Suzanne Ahlstav Hernandez, course administrator
Gunilla Tibbling, course administrator

Administration

Anna Backeryd Lindström, accounting
Pirkko Boox, senior advisor
Jenny Djerf, accounting
Ulf Dohlmar, accounting
Birgitta Gustafsson, financial coordinator
Holger Henningsson, personnel
Kerstin Henriksson, communication
Katarina Israelsson, accounting
Christina Magnusson, administrator of postgraduate education
Sara Mulder, personnel
Barbro Nelson, accounting
Camilla Nilsson, personnel coordinator
Klara Nilsson, personnel
Helene Norlin, adm. assistant
Camilla Sandgren, accounting
Tuulikki Simu, accounting
Ulla Steimer, accounting

Core Facilities

BioVis

In 2010, the former Cell Analysis Core Facility was reshaped to create BioVis, a regional resource for advanced visualization and analysis of biological material. The BioVis Facility is part of Science for Life Lab since 2010.

BioVis provides services and instrumentation for Electron and Light microscopy, flow Cytometry, cell sorting and Image Flow Cytometry. Researchers from Academia as well as non-Academia users are welcome to analyze their own samples on the instruments available in the lab. We provide hands-on training and advice for handling and we offer advice on the use of appropriate methods and experimental setups. For users who wish to have this service provided we can, time permitting, also perform sorting and analysis experiments.

Instrumentation at BioVis includes a FACS Aria III flow sorter and a BD LSR Fortessa multilaser flow cytometer as well as an Merck/AMNIS Flowsight Imaging Flow Cytometer. (first in Scandinavia). A ZEISS LSM 700 confocal microscope, a ZEISS 710 NLO multiphoton microscope and ZEISS 710 Superresolution SIM and a ZEISS AxioImager brightfield and fluorescence microscope are also installed. BioVis is proud to be first in Scandinavia having a ZEISS Lightsheet Z.1 microscope installed. In addition, users have access to workstations for image analysis and documentation including IMARIS and Huygens software. BioVis is collaborating with the group of C. Wählby, Centre for Image Analysis (CBA), for in-depth image analysis.

An FEI Tecnai Biotwin transmission electron microscope has also been added to the facility. This instrument has increased our capacity to provide the services requested by various research groups. A laboratory and staff to prepare samples for imaging on the electron microscope is available.

The service level is high with a lab manager and experts for microscopy, flow cytometry, electron microscopy and image analysis available to instruct and advise users, to ensure instrument performance, to perform experiments and to administrate instrument service and reservations.

In 2015 BioVis served a total of 173 projects, with the majority (80.9 %) coming from the Disciplinary Domain of Medicince and Pharmacy, Uppsala University, followed by companies (6.9 %), Disciplinary Domain of Science and Technology, Uppsala University (5.2 %), Swedish University of Agricultural Sciences, Lund University (3.5 %) and Stockholm University/Karolinska Institutet (2.9 %). Within the Medicince and Pharmacy, research groups, IGP based research groups made up for 50 % of the projects.

BioVis restructured its highly appreciated Course “Methods for Cell Analysis” (MCA) into a 1.5 week course with its strength in in-depth lectures and hands-on sessions on various instruments and software BioVis can offer. We also established a new course “Introduction to Image Analysis Software” (IAS), to meet the needs of customers to get started and introduced to image analysis using different software. Both courses gained quickly a very good reputation for its quality and we received applications from all over Sweden. The MCA and IAS courses are held now twice a year, spring and autumn.

To meet demands in Instrument introduction BioVis gives monthly introductory courses on its instruments, again covering the techniques available by lectures and hands-on session.

Another monthly held course “First steps in Image Analysis” is open to everybody to get started on the free Image analysis software “ImageJ”.

BioVis is organizing on a regular basis workshops and symposia. To meet its standards BioVis started to form a BioVis Advisory Board, which was installed in 2015. Christer Betsholtz took over the position as BioVis director from Lena Claesson-Welsh.

Staff 2015
Christer Betsholtz, facility director
Lena Claesson-Welsh, facility director
Dirk Pacholsky, head of facility
Anders Ahlander, research engineer
Matyas Molnar, research engineer
Sara Peterson, research engineer
Kenneth Wester, research engineer
Jeremy Adler, research engineer

Clinical Sequencing Facility

In 2013, Uppsala University, Uppsala University Hospital and Science for Life Laboratory together formed a new facility at the department of Immunology, Genetics and Pathology: the Clinical Sequencing Facility. The mission of the facility is to provide services for high throughput genomics in real clinical applications, from new genetic tests in routine diagnostics to translational research projects based on next-generation sequencing (NGS). The facility is one of three facilities constituting the national SciLifeLab platform for Clinical Diagnostics.

The facility is organized into three diagnosis-oriented work-packages: Solid tumours (WP1), Hematological malignancies (WP2), and Inherited conditions (WP3). A separate work-package is dedicated for bioinformatics (WP4) to secure a rapid and accurate handling and storing of clinical NGS data. A fifth work-package focuses on ethical aspects and reporting of NGS data in the clinical context.

We aim to have stable, high-quality sequencing equipment that enables us to perform a wide range of premium clinical assays. We currently have two Illumina MiSeq, one NextSeq and one HiSeq instrument and will buy a second NextSeq and a MiniSeq instrument during 2016. In addition, we take advantage of long-read PacBio sequencing available through NGI, Uppsala, and, as the first facility in Sweden, offer a clinical test based on this technology.

We offer a number of tests and services in our diagnostic areas, such as gene panel tests for inherited cardiac and connective tissue diseases that are now used in routine practice at Clinical Genetics, Uppsala University Hospital. These tests are also accredited with ISO 15189. Reduced and clinical exomes are also in routine practise, and full exomes later in 2016. We also offer diagnostic gene panel tests for colon and lung cancer, melanoma, and gastrointestinal stromal tumors (GIST). These panels are now in production at Uppsala University Hospital, Molecular Pathology, and are designed for formalin-fixed paraffin-embedded (FFPE) tumor material. We are also providing a number of gene panel based tests for different types of leukemia.

We collaborate actively with the facilities within National Genomics Infrastructure (NGI) on NGS-based tests for clinical diagnostics that use technology other than what is installed in our own facility. In particular, whole-exome sequencing with HiSeq instruments, and long-read sequencing with PacBio, are important parts of this. We also collaborate with UPPMAX, the Uppsala facility for high performance computating, which is part of SNIC, the Swedish National Infrastructure for Computing, and the European Bioinformatics Institute (Hinxton, UK) on sequence data management.

The facility became the Swedish country node for the UNESCO-protected international Human Variome Project in 2014.

Staff 2015
Richard Rosenquist Brandell, facility director
Johan Rung, head of facility
Johan Botling, work-package leader, solid tumors
Lucia Cavelier, work-package leader, hematological malignancies
Marie-Louise Bondeson, work-package leader, inherited diseases
Lotte Moens, molecular geneticist
Britt-Inger Jonsson, BMA
Eva Saarinen, BMA
Tatjana Pandzic, molecular geneticist
Elin Falk Sörqvist, bioinformatician
Malin Melin, bioinformatician
Claes Ladenvall, bioinformatician
Patrik Smeds, bioinformatician

NGI-Uppsala/Uppsala Genome Center

The Uppsala Genome Center (UGC) is one node of the National Genomics Infrastructure (NGI), and has been established by the Swedish Research Council (VR) and is hosted by SciLifeLab Sweden. The facility is open to academic research groups in Sweden on a non-profit basis. Our vision is to provide tailor-made, cost-effective and expedient solutions for all types of genetic/genomic projects using the Massively Parallel Sequencing (MPS) technologies of Ion Torrent (Thermo Fisher Scientific) and SMRT sequencing (Pacific Biosciences), as well as Sanger sequencing and STR typing, thus contributing to the broad spectrum of services offered at NGI.

The services offered by UGC are:
1. Massively Parallel Sequencing (MPS) on Ion Torrent (PGM™), S5XL, Ion Proton™ systems from Life Technologies and on RSII from Pacific Biosciences.
2. Sanger Sequencing Service
3. Genotyping with STR-markers
4. Service for separation of custom prepared samples by capillary electrophoresis on AB3730XL Genetic Analyzer

The MPS technologies can be used for *de novo* sequencing, whole genome re-sequencing and targeted re-sequencing of DNA. RNA sequencing can be performed either as whole transcriptome analysis, gene expression profiling, or as sequencing of small RNA molecules. UGC can offer different MPS technologies for different type of projects, with variation in output of sequencing data from 10 Mbp till 1000 Mbp per run and read length from 200 bp to 60 000 bp.

Besides the sequencing service, the facility is engaged in technology development and scientific collaborations aimed at the advancement of novel methods and applications of MPS. In particular, UGC has a number of projects together with clinical genetics and clinical immunology at Uppsala University Hospital to promote the application of rapid, high-throughput MPS sequencing in translational medicine. UGC also plays an important and increasing consultative role in guiding scientists in the design of sequencing projects and choice of suitable technology. We also participate in a number of educational and outreach activities of Swedish academic users on MPS and methods of data analysis.

In 2015 UGC sequenced and delivered data from 224 MPS projects to 101 unique users. 150 researchers are more or less frequent users of the Sanger Sequencing Service and the other types of services that UGC offers.

Staff 2015
Ulf Gyllensten, facility director
Inger Jonasson, head of facility
Adam Ameur, bioinformatician
Magdalena Andersson, research engineer
Ulrika Broström, research engineer
Ignas Bunikis, bioinformatician
Nicola Cahill, research engineer
Susana Häggqvist, research engineer
Ida Höijer, research engineer
Carolina Ilbäck, research engineer,
Sebastian Johansson, research engineer
Cecilia Lindau, research engineer
Anne-Christine Lindström, research engineer
Anna Petri, research engineer
Maria Schenström, research engineer
Christian Tellgren-Roth, bioinformatician
Olga Vinnere Pettersson, project coordinator
Nina Williams, research engineer

PLA Proteomics Facility

The PLA Proteomics facility that is part of the Affinity Proteomics platform of Science for Life Laboratory (SciLifeLab), was established in 2010 and provides services for the scientific community for sensitive and specific analyses of proteins and their interaction complexes using *in situ* proximity ligation assays (*in situ* PLA). Since 2012 the facility also assists users by establishing solid-phase PLA tests for sensitive and specific detection of proteins in body fluids such as plasma, cerebrospinal fluids, etc. The services further include high-performance PLA-based western blot assays.

The PLA technology was developed at the Department of Immunology, Genetics and Pathology, Division of Molecular Tools, and allows target protein molecules to be sensitively analyzed using sets of antibodies with conjugated oligonucleotides. Upon recognition of target molecules by the antibodies, the attached oligonucleotides can either be ligated to each other (for solution-phase PLA), or guide circularization of two accessory oligonucleotides (for *in situ* PLA). The reporter DNA molecules that form by ligation are amplifiable by real-time PCR for solution-phase measurements or by localized rolling circle amplification for *in situ* detection. The PLA method owes its specificity and sensitivity to the requirement for multiple recognition events and the possibility of translating the detecting signals to amplifiable DNA reporters.

During 2015 the facility offered Swedish scientists both service for fee and also participated in collaborative projects. A large and growing number of *in situ* PLA-based assays are available for analyses of proteins in cells and tissues at single cell and single molecule resolution. The facility can also establish assays for new target molecules or adapt established assay formats for new applications, by mutual agreement with users. The assistance also includes expert advice on design of experiment and for data analyses.

Staff 2015
Ulf Landegren, facility director
Masood Kamali-Moghaddam, head of facility
Radiosa Gallini, research engineer
Agata Zieba, researcher

Single Cell Proteomics

The SciLifeLab Single Cell Proteomics Facility offers services to the scientific community to analyse proteins, or both RNA and protein, in a highly multiplex manner (n = 24-96) in single cell. The Facility offers validated human cellular protein panels (in collaboration with Olink Proteomics), custom protein panels (autumn 2016), support to set-up multiplex RNA gene expression assays in single cells, and guidance for experimental design, downstream QC, and data analysis.

We work with the BioVis Facility for single cell sorting into 96 or 384 well plates. We can analyze cells isolated from cell lines or tissues, and our methods can be used for single cells or several hundreds of cells.

Staff 2015
Ulf Landegren, facility director
Caroline Gallant, head of facility
Marcus Danielsson Fernow, research engineer
Hongxing Zhao, research engineer

Tissue Profiling Facility

The Tissue Profiling Facility was established during 2010 as part of the SciLifeLab effort in Uppsala. In 2013 it became a national facility within the SciLifeLab Affinity Proteomics platform. The expertise of the centre is focused on histopathology with special technical emphasis on tissue microarray (TMA) production, immunohistochemistry (IHC) and image digitalization of stained slides (scanning). As a technical high-throughput platform, the centre aims to provide these services to external research groups. During 2015 the facility performed service for researchers that included the construction of 52 TMAs, 14,000 cut tissue sections, 6000 slide scannings and 4300 IHC or H/E-stained slides.

The origin of the facility builds on more than a decade of accumulated experience and know-how from being a central part of the Human Protein Atlas project. This project which is funded by the Knut and Alice Wallenberg research foundation, is set up to map the human proteome by generating and validating antibodies to be used for high throughput protein profiling of normal human tissues, different forms of cancers and multiple cell lines. Tissue microarrays are constructed using four different systems; a fully automated TMA production system (TMA GrandMaster), an automated system (Beecher ATA-27), a semi-automated system (Pathology Devices) and a manual arrayer (Beecher MTA-1) depending on tissue used and amount of tissue available. Sections are cut using a waterfall microtome (Microm HM355S).

Immunohistochemistry is performed in an automated slide staining system (Lab Vision Autostainer 480) on formalin fixed paraffin embedded material, using a polymer based detection system. Slides are deparaffinized and dehydrated in an automated slide staining

system (Leica Autostainer XL) and mounted in an automated glass cover slipper system (Leica CV5030).

By using bright field digital scanners based on line scanning technology (Aperio Scanscope XT and AT), stained glass slides are transformed to digital images. Images are subsequently exported and up-loaded to a server for viewing. Slides are scanned using 20x or 40x magnification. The high-resolution images can be viewed using a freely available software (ImageScope) from Aperio.

Staff 2015
Fredrik Pontén, platform director
Per-Henrik Edqvist, head of Facility
IngMarie Olsson, technician
Dennis Kesti, technician
Erik Lindahl, technician
Lillemor Källström, technician
Maria Aronsson, research engineer

Prizes and awards

Per Westermark was awarded the **Hilda and Alfred Eriksson Prize** for his research on amyloid diseases.

Taija Mäkinen received the prize **Eric K Fernströms Svenska Pris** for her renowned research on the formation of lymphatic vessels.

Hannah Karlsson was awarded **the Hwasser prize** for best pre-clinical PhD thesis.

Hadis Honarvar received the **Marie Curie Award** for her abstract submitted to the conference *Annual Congress of the European Association of Nuclear Medicine.*

Undergraduate Education at IGP

The Department participates in the education programs in Medicine, Biomedicine, Biomedical Laboratory Science and Physiotherapy. The master programs in Forensic Science, Molecular Medicine and Medical Nuclide Techniques are organized by IGP.

Medicine

We participated in the courses “Growth and Degeneration” in the second semester, "Attack and Defense” in the fourth semester, and in the integration periods in the fourth, fifth, sixth, seventh and eighth semester of the medicine program. Students on the sixth semester also had a clinical rotation "Clinical Pathology". A two-week course in Cancer Genetics and Tumour Biology was given on the seventh semester, as well as a clinical rotation in Oncology. Approximately hundred students attended the different courses, which are given twice a year.

Biomedicine

In this program we gave a 7.5 credit course in Medical Genetics. This course is given during the fourth semester of the Biomedicine program. About thirty-five students participated in the course. We also teach general pathology in the course "Diseases - Clinical Survey" on the sixth semester of the program.

Biomedical Laboratory Science

During the fall we gave a course in Pathology and Clinical Genetics within the Biomedical laboratory science program. This course is for 11 credits and is given to students in the third semester of their education. We also headed two other courses at the program during the spring semester "Immunology and Transfusion Medicine 12 credits" (fourth semester on the program) as well as "Advanced Course I 7.5 credits" (sixth semester). Approximately fifty students attended each course.

Single Subject Courses

We offer a web-based course in Basic Medical Genetics. The course is for 4.5 credits and was given twice during 2015. It was completely web-based, with lectures, study questions and exam available via the PING-PONG platform. Students from all over Sweden, as well as abroad, enroll in this course.

Three courses, “Medical Genetics 7.5 credits”, "Immune, Gene and Cell Therapy 7.5 credits" and “Molecular Mechanisms in Cancer 7.5 credits”, were given during the fall. The course “Medical Technology” was given as an elective course for students in the Master Programme in Engineering Physics. Several of the courses given in our international master programs were also open as single subject courses.

Master Programs

The Department is heading three international master programmes. The master programme in Forensic Science is based on knowledge from leading international research and is closely linked to research in the field. Courses in Medical Genetics, Forensic Science and Criminalistics, Forensic Genetics and Medicine, Criminology, Forensic Chemistry and Analytical Toxicology are included. In addition to IGP, other departments at Uppsala University and Stockholm University are arranging courses within the programme. Approximately twenty students are enrolled on each occasion.

The international master programme in Molecular Medicine has twenty-five student positions. The programme is focused on molecular mechanisms causing diseases and new

technologies in genomics, epigenetics and proteomics. The courses in Medical Genetics and Cancer, and Advanced Techniques in Molecular Medicine, are given on the first semester of the programme. Courses in Epigenomics, Biomarkers, Bioimaging and Regenerative Medicine are included in the programme on the second and third semester.

The third international master programme Medical Nuclide Techniques focuses on medical applications of radionuclides. The programme provides both theoretical and practical teaching. Examples of courses the first year of the programme are Radiation Protection and Medical effects, Nuclide Production and Radiochemistry, as well as Good Manufacturing Practice (GMP). The second year a course in Labelling Chemistry and Compound Development is given.

In an evaluation performed by the Swedish Higher Education Authority a few years ago all three master programmes organised by IGP received the highest credentials "very high quality".

Postgraduate Education at IGP

In 2015 the Department had 117 students registered for a postgraduate education. Fifteen PhD students defended their PhD theses and three students obtained licentiate degrees.

Postgraduate education at IGP is performed as main scientific work in research groups under the guidance of at least two supervisors. Postgraduate studies also require participation at postgraduate courses. The Department encourages postgraduate students to attend international courses and has allocated funds from which students can apply for funding to participate in such courses, or to visit research laboratories to learn techniques required for their research projects. In 2015, four students received this type of funding.

The graduate students at IGP organized a conference in Visby, Gotland 4-6 June, 2015, with invited speakers and where many students presented their work in oral presentations and on posters.

Postgraduate courses

At the Rudbeck Laboratory, the BioVis facility organises the postgraduate student course "Methods in Cell Analysis". The course contents include fluorescence theory, basic and advanced confocal microscopy and flow cytometry. The students also have the opportunity to try out suitable methods in their own projects.

Another postgraduate course given at the Rudbeck Laboratory is "Advanced Molecular Technology and Instrumentation for Proteome Analyses", which is organized by the proteomics platforms at Science for Life Laboratory in Uppsala.

The course "Towards individualized cancer therapy" was organized in collaboration with U-CAN, a facility for cancer research supported by the Swedish Government. Some students also follow "NatiOn", which is a national school for graduate students in clinical cancer research held at Karolinska Institutet in collaboration with Uppsala University, including teachers from IGP.

All postgraduate students attend a pedagogical course given at Uppsala University.

Seminar Series

The Rudbeck Seminar Series, organized by IGP, was given as a course for PhD students in both the spring and fall semesters. PhD students who regularly attend the seminars can account for three credit points per semester in their PhD education. The seminars are held by

invited speakers from other Swedish universities as well as from abroad, on topics relevant for the PhD students at the Department. In 2015, 34 seminars were given in the series.

Some students working in IGP laboratories at the Biomedial Centre (BMC) also attended the SciLifeLab/The Svedberg seminar series held there.

The course “Frontiers in biomaterials and regenerative medicine” is a seminar series organised by IGP, focusing on principles and methodologies associated with biomaterials, cells and strategies for tissue regeneration.

Clinical and Experimental Pathology

Research projects within the programme *Clinical and experimental pathology* focus on disease related alterations to be observed in the tissue. The main objectives are to understand pathogenesis, develop diagnostics further, identify potential targets for new therapies and look for new not previously known alterations. We study both morphological and molecular alterations. e.g. in protein expression or on the DNA or RNA level. On-going projects include studies on tumours, inflammation and degeneration to be observed in various organs.

Neuropathology

Irina Alafuzoff

The research carried out focuses on various degenerative processes, degenerative diseases and tumours of the human brain. The material that is studied is obtained from humans, brain and other organs, obtained post-mortem (neurodegeneration and vascular pathology) or during surgical procedure (primary brain tumours). All studies on human tissue are carried out following the current legislation in Sweden; The Act (2003:460) and the statute (2003:615) concerning the Ethical Review of Research Involving Humans; the statute (2007:1069) with instructions for Regional Ethical Review Boards; the statute (2007:1068) for the Central Ethical Review Board. The translations of the Act (2003:460) and the Statute (2003:615) are updated with changes that came in to force 2008).
The methods applied are various and include among others histology, immunohistochemistry and *in situ* hybridization.

Neurodegenerative diseases

Adila Elobeid, Svetlana Popova Maria Leino P, Tuomas Rauramaa (Kuopio, Finland)

One of the major events in neurodegeneration is misfolding of proteins that tend to accumulate in the cells or matrix. Misfolding of proteins increases with aging. Accumulation of misfolded proteins leads to functional disturbances seen as various movement disorders or cognitive impairment/dementia.

Based on current knowledge the most common form of neuronal degeneration is the hyperphosphorylation of the tau (HPtau) protein followed by alteration of beta-amyloid (Aβ), alpha-synuclein (αS) and transactive response DNA binding protein 43 (TDP43).

The questions addressed by the research team during 2015 are briefly the following; initiation site of neuronal degeneration, incidence of various types of neuronal degeneration to be seen in the aging population, progression pattern, associated alterations such as astrogliosis, microgliosis and seeding of misfolding of proteins.

Primary brain tumors

Sylwia Libard

The most devastating brain tumour is glioma that can be of various grades ranging from I to IV. Currently there is no cure for these tumours and the most malignant glioma of grade IV is lethal. Treatment strategies include surgery, radiotherapy and chemotherapy. The main focus today is to identify new treatment strategies. For this approach detailed assessment of tumours, i.e., morphology, protein expression and molecular data is required. The question addressed by the research team is briefly the following; alteration in protein expression in relation to grade, neuroanatomical region, recurrence and treatment. The project is preformed in collaborations with neurosurgeons, radiologists, oncologists and basic scientists.

Vascular brain pathology

Yasmin Lundström, Patrik Lundström

With aging the cardiac function as well as the vessels display age related changes that ultimately lead to various extent of circulatory failure. Brain tissue alterations related to insufficient circulation are common but poorly investigated. A brain infarct can be seen as a defined lesion. Assessing brain tissue with diffuse neuronal loss, loss of oligodendrocytes or activation of astrocytes or microglia that is initiated by various severity of ischemia/anoxia is more difficult.

The question addressed by the research team is briefly the following; primary protein alteration to be seen in neurons, astrocytes, oligodendrocytes or microglia at hypoxia/anoxia.

Pilot studies have been carried out involving the Sofosko students Yasmin Lundström and Patrik Lundström.

Members during 2015

Irina Alafuzoff, professor, group leader
Adila Elobeid, PhD student
Maria Leino, PhD researcher
Svetlana Popova, researcher
Sylwia Libard, PhD student
Tuomas Rauramaa, PhD student,
Yasmin Lundström, Sofosco student medicine
Patrik Lundström, Sofosco student medicine

Funding during 2015

ALF, 1 067 kSEK
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United Nations Educational, Scientific and Cultural Organization, fellowship to Adila Elobeid

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Translational Tumor Pathology

Patrick Micke – Johan Botling

The Molecular Pathology of Non-small Cell Lung Cancer

Non-small cell lung cancer (NSCLC) represents a histologically mixed group of highly aggressive tumors. In subsets of patients, distinct genetic aberrations have been identified that are now successfully exploited for therapeutic intervention. However, for the vast majority of patients, treatment options are scant and overall prognosis poor. The molecular characterisation of NSCLC is challenging because of its notorious heterogeneity and its apparent genetic instability. Correlation of molecular alterations in the tumor tissues of individual patients to actual clinical outcome is essential in order to understand the basic tumor biology, and for development of diagnostic biomarkers and new treatment strategies.

In an explorative phase we have investigated fresh frozen tissue samples of consecutively operated NSCLC patients and obtained comprehensive molecular landscapes by the use of array technology, sequencing methods, tissue microarrays and immunohistochemistry. The combined clinical, histopathologic and molecular data set represents the largest single institute cohort of this kind worldwide and forms the vantage point for translational studies. We have identified specific aberrations on genomic and transcriptomic levels that are strongly associated with clinical outcome. Using immunohistochemistry these molecular changes were confirmed on the protein level in independent cohorts, thus, have potential for use in clinical diagnostics (Micke et al., 2011; Botling et al., 2013).

In addition to epithelial tumor cell characteristics, stromal components were identified and correlated to relevant patient characteristics and survival (Edlund et al., 2012). Immunoglobulin light chain expression and plasma cell infiltration were demonstrated as powerful prognostic markers in NSCLC and other human solid tumors (Lohr et al., 2013; Schmidt et al., 2012). The results highlight the impact of the host's immune response in tumorigenesis. To identify potential immunogenic targets we applied RNA sequencing technology on 204 NSCLC tumor samples. This analysis provided unexcelled resolution of gene expression, including splice variants and mutations. The combination of our NSCLC data set with 32 different normal tissues (Lindskog et al., 2014) allowed characterization of lung cancer specific gene expression. Based on this data we were able to define the landscape of cancer testis antigens in NSCLC on the transcriptomic and proteomic level, hopefully, providing new cancer specific targets for immunotherapeutic intervention (Micke et al., 2014).

International collaborations have resulted in a number of publications over the last years, including the landmark genomic characterization of small cell lung cancer conducted by a consortium led by the Cologne translational oncology group (George et al., 2015).

Translation to diagnostic molecular pathology

A key effort of our group is to translate knowledge and established technology developed in research projects into routine diagnostics. To this end, somatic mutation assays for cancer specimens (KRAS, NRAS, BRAF, EGFR and PIK3CA) have been implemented at the molecular pathology unit at the clinical Department of Pathology. Our group now leads the Solid Tumor Work Package (WP.1) in the national Clinical Sequencing Platform (Science for Life Laboratory). The development of targeted NGS and linked bioinformatic pipelines adapted to formalin-fixed paraffin-embedded cancer biopsies (Moens et al., 2015) has led to the launch of multiplex diagnostic mutation assays for colon cancer (2014), lung cancer (2015), melanoma and GIST (2016) in routine health care. The goal is to provide cutting edge cancer diagnostics to patients in our region and nationally through clinical collaborations. Full

population-based comprehensive diagnostic coverage (Sandelin et al., 2015) forms the basis for fair and equal cancer care and provides a foundation for inclusion of patients with specific alterations into clinical and translational research.

Group members during 2015

Patrick Micke, associate professor, group leader
Dijana Djureinovic, PhD student
Johanna Mattsson, PhD student

Johan Botling, associate professor, group leader
Johan Isaksson, PhD student
Linnea LaFleur, PhD student
Lotte Moens, PhD, project leader, ClinSeq platform
Magnus Sundström, PhD, researcher
Elin Falk-Sörqvist, Bioinformatician

Funding 2015

Patrick Micke
Swedish Cancer Society, 500 kSEK
Regional Research Council (Uppsala-Örebro region), 300 kSEK
ALF, 500 kSEK
Lions Cancerforskningsfond Uppsala, 200 kSEK
Selanders Stiftelse Uppsala, 150 kSEK

Johan Botling
Swedish Cancer Society, 500 kSEK
Regional Research Council (Uppsala-Örebro region), 400 kSEK
ALF, 800 kSEK
Lions Cancerforskningsfond, 150 kSEK
Gävleborg Region, 675 kSEK
CFUG Gävle, 135 kSEK
Gävle Cancerfond, 400 kSEK

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Amyloid Research

Per Westermark

The assembly of proteins into amyloid fibrils as cause of disease is attracting increasing attention, not only in systemic disorders and in connection with neurodegenerative conditions but also associated with other diseases such as type 2 diabetes. We have a broad interest in the nature, pathogenesis and impact of a number of amyloid diseases, both systemic and localized.

Together with researchers in Umeå we have found that there are two distinct phenotypes in Swedish familial transthyretin (TTR)-derived amyloidosis and that these are characterized by differences in posttranslational processing of the protein. We can distinguish between the two with the aid of a simple subcutaneous adipose tissue biopsy. This is important since one of the phenotypes carries a big risk of progressive cardiomyopathy also after liver transplantation, which is the main treatment today. While the Swedish type of mutation (V30M) is characterized by the two different phenotypes, most other TTR mutations have the phenotype associated with a risk for cardiomyopathy. We have recently shown that spinal stenosis may be a manifestation of TTR-amyloidosis, both of wildtype and of mutation-associated type. A program to study this is in progression.

The possible transmission of amyloid diseases by a prion-like mechanism is one of our main interests. We are, in collaboration with researchers at SVL and SLU, Uppsala, performing studies on the possibility that AA-amyloid may be present in our environment including our food and act a putative risk factor for development of the disease in animals and human. Together with G.T. Westermark, Department of Medical Cell Biology, we have found that seeding, cross-seeding and transmission of localized amyloidoses are possible, such as those consisting of Aβ and IAPP.

Localized amyloid has been identified as important actors in Alzheimer's disease and type 2 diabetes. We are currently investigating the possibility that amyloid deposits also are important in some other major diseases, particularly aortic aneurysm and atherosclerosis. Amyloid in atherosclerotic plaques is an overlooked phenomenon and our hypothesis is that toxic protein aggregates are mechanistic in the pathogenesis of atherosclerotic lesions. We are evaluating a candidate protein for the atherosclerotic amyloid fibril.

Our laboratory is also working in association with the University Hospital and is performing amyloid diagnostic work within the hospital. As systemic amyloidoses are increasingly identified as clinical problems particularly in elderly, we are receiving an increasing number of biopsies each year. Our laboratory is devoted to further develop existing methods to determine type of systemic amyloidosis. For this, we are also developing new antibodies for clinical use and are planning to introduce mass spectrometry.

Group members during 2015

Per Westermark, professor em., group leader
Ellahe Charkhkar, lab technician

Funding during 2015

Selanders stiftelse, 100 kSEK

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Clinical Pathology

The objective in different research projects carried out at the section of clinical/surgical pathology is to increase knowledge regarding disease related alterations observed in tissue. One goal is to improve diagnostics to make it more informative, another is to identify potential targets to be used for the development of new treatment strategies. The alterations to be looked for can be seen as morphological changes, changes in protein expression or on the DNA or RNA level.

The assessed tissues are obtained from humans, i.e., biopsies, surgical specimens or autopsy specimens and the methods implemented are numerous. All studies on human tissue are carried out following the current legislation in Sweden; The Act (2003:460) and the statute (2003:615) concerning the Ethical Review of Research Involving Humans; the statute (2007:1069) with instructions for Regional Ethical Review Boards; the statute (2007:1068) for the Central Ethical Review Board. The translations of the Act (2003:460) and the Statute (2003:615) are updated with changes that came in to force 2008).

Hematopathology

Rose-Marie Amini, Christer Sundström, Maysaa Abdulla, Peter Hollander

Inflammatory cells are in close proximity to all kinds of malignant tumours, where the innate immunity comprise myeloid cells acting as the first line of defence. The innate immunity includes macrophages, granulocytes (neutrophils, eosinophils, basophils), mast cells, dendritic cells and NK cells. The adaptive immunity (lymphoid) is the acquired immunity consisting of B- and T-cells.

Our group studies the microenvironment in malignant lymphomas with a special focus on the inflammatory cells in Hodgkin lymphoma and diffuse large B-cell lymphomas (DLBCL). The presence and function of the surrounding immune cells are correlated in large population-based patient cohorts to clinical data like patient characteristics, treatment outcome and survival. In addition, the composition of immune regulatory cells in peripheral blood is studied in correlation to treatment outcome and characeristics in the lymphoma tissue.

We also focus on the pathogenetic mechanisms in B-cell lymphomas like diffuse large B-cell lymphoma (DLBCL) and marginal zone lymphomas focusing on the localisations in the central nervous system and spleen. Further, some studies involve next generation sequencing of T-cell lymphomas aswell, like T-prolymphocytic leukemia.

Malignant melanoma

Margrét Agnarsdóttir

The incidence of cutaneous malignant melanoma has increased dramatically in Caucasians in the last few decades, an increase that is partly explained by altered sun exposure habits. For the individual patient, with a localized disease, the tumour thickness of the excised lesion is the most important prognostic factor. However, there is a need to identify characteristics, especially for patients with thin melanomas (< 1mm) that can place patients into certain risk groups.

The protein expression of multiple proteins in malignant melanoma tumours has been studied, with the aim of identifying potential new candidate biomarkers. Representative samples from melanoma tissues have been assembled in a tissue microarray format and protein expression studied using immunohistochemistry. The majority of the tumour samples studied are primary melanoma tumours from two cohorts, however a new cohort with both primary and metastatic tumours has been built. The primary tumour cohorts have also been employed to develop an

automated algorithm to identify melanoma cells in the tissue samples. It has been difficult to identify new single prognostic protein markers that have a stronger predictive value than the thickness but some of the markers were described for the first time in melanomas. However, combining results for a few markers employing the automated algorithm has revealed interesting combination of markers that we are still working with.

Pituitary gland disorders

Olivera Casar-Borota

Pituitary adenomas represent more than 90% of pathological lesions in the pituitary gland and constitute about 10 to 15% of all intracranial neoplasms in adults. .

After a series of papers published on the topic of predictive and prognostic factors in growth hormone-producing adenomas and corticotroph adenomas, we are now focusing our research on biomarkers of clinically non-functioning pituitary adenomas (NFPAs). Although NFPAs are hormonally inactive, they express pituitary hormones in the tumour cells, most commonly gonadotroph hormones, and occasionally other hormones, and can be classified in different hormonal subtypes according to the hormone expression. We are going to examine how growth pattern of NFPAs correlate with the hormonal subtypes of the tumours in a large cohort of more than 250 patients. We will further explore the expression of somatostatin receptors type 1, 2, 3 and 5 in NFPAs in order to examine whether patients with NFPAs could be candidates for medical treatment with Pasireotide, a new somatostatin analogue with a broad affinity for multiple somatostatin receptor types. Moreover, we will analyse the expression of E-cadherin, a marker of epithelial-mesenchymal transition and to correlate the E-cadherin expression with the growth pattern and local invasiveness of the NFPAs. The project on NFPAs is a collaborative project with Prof Jens Bollerslev and his research group from Oslo University.

Another project addresses inflammatory disorders of pituitary gland with special focus on recently described IgG4-related hypofysitis (IgG4-RH). This is probably underestimated disorder and more research on the topic is important to increase the awareness of this treatable disorder and to improve an early diagnosis. We aim to collect cases of the IgG4-RH from the surgical material from Uppsala University Hospital and to correlate histopathological changes with MRI findings and clinical data. We will specially focus on additional histopathological changes in IgG4-RH specimens that can help us to understand the mechanisms triggering IgG4-reaction in the pituitary gland. This project involves Dr Sengul Ahlstrom, a trainee at the Pathology Department and represents a collaboration with the Endocrinology Dept. (Doc Dr Britt Edén-Engström), Dept. of Radiology (Adj Prof Johan Wikström) and Dept. of Neurosurgery (Dr Olafur Gudjonsson) at Uppsala University Hospital.

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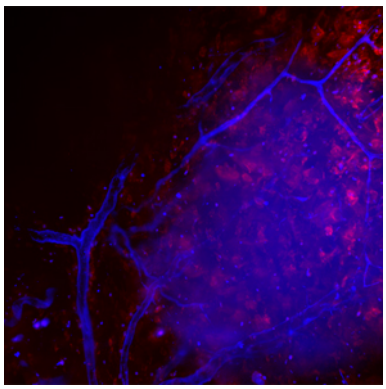
Clinical Immunology



The Clinical Immunology research groups have a strong translational focus. The research projects aim to increase the understanding about immunological mechanisms in patients with cancer or autoimmune disease (diabetes, rheumatoid arthritis, systemic lupus erythematosus or multiple sclerosis) and to explain the immune reactions that occur when immune cells or components come in contact with biomaterial, transplanted organs, cells or viruses used for therapy.

Within this research area we are developing novel immune, gene and cell therapies as well as diagnostic/prognostic markers, which are tested in clinical trials in collaboration with Uppsala University Hospital, other national and international universities, the immune diagnostic industry, EU networks and the Nordic Network for Islet Transplantation.

Gene, Cell and Immunotherapy of Cancer

Magnus Essand

Immunotherapy is currently being established as cancer treatment. Immune checkpoint blockade antibodies that fine-tune T cell activity to kill tumor cells can induce long-term remission in up to 50% of patients with refractory melanoma, lung cancer and kidney cancer. Adoptive transfer of patient-derived T cells that are engineered *ex vivo* to express a chimeric antigen receptor (CAR) results in complete remission in a majority of patient with refractory B cell acute lymphoblastic leukemia. Oncolytic vaccines, which are replication-competent viruses engineered to selectively kill tumor cells and deceive the immune system to believe that the tumor is a foreign entity that needs to be eradicated are emerging as the next breakthrough in cancer immunotherapy.

Although successful in part, immunotherapy has so far not delivered for most forms of cancer. It is apparent that the technologies need to be improved and refined further to optimize treatments. We are focusing on translational cancer immunotherapy research specializing on virus, dendritic cell (DC) and T cell modifications to develop new potent anti-cancer agents. Some virus and T cells developed in our laboratory are already in clinical phase I trials and others are about to enter clinical trials.

Oncolytic virus therapy

Viruses are genetically engineered to selectively kill tumor cells and induce a potent and adequate anti-tumor immune response. Virus infectivity is altered through genetic modification of the virus capsid to favor infection of tumors cells. Virus replication is altered by introduction of regulatory elements, such as promoter and/or microRNA target sequences into the virus genome to specifically control viral gene expression to tumor cells. Progeny virus, produced upon virus replication in tumor cells, can infect neighboring tumor cells, thus amplifying the initial inoculums. The lytic cell death induced by virus is not dependent on the ability of the tumor cell to go into apoptosis, thus also drug-resistant cancer stem cells can be

killed. Furthermore, the presence of immunogenic virus in the tumor microenvironment can alter the otherwise immunosuppressive milieu in favor of an anti-tumor immune response. To further boost adequate anti-tumor immune responses the virus encodes a transgene, such as *Helicobacter pylori* neutrophil-activating protein (HP-NAP), for a Th1-type immune activation.

In order to target metastatic sites we are evaluating various cell types as carriers to deliver oncolytic virus at the tumor site. In particular, we are evaluating macrophages as virus carries, in collaboration with Claire Lewis at Sheffield University, as macrophages are attracted to inflammatory cancer environments. We focus our efforts on prostate cancer, neuroendocrine cancer and neuroblastoma and we are primarily working with oncolytic adenovirus but also Semliki Forest virus and Vaccinia virus.

T Cell therapy

T cells are genetically engineered to express novel T cell receptors (TCR) or chimeric antigen receptors (CAR) that recognize antigens that are expressed and presented by u cells. This way the engineered T cells specifically target and kill tumor cells. We have recently cloned a TCR against a prostate tumor-associated antigen called TARP and shown that genetically engineered T cells expressing this TCR can selectively kill prostate and breast cancer cells. We are also developing CAR T cells targeting PSMA on prostate cancer cells, GD2 on neuroblasoma or CD19 on B cell malignancies.

Besides developing new CAR transgenes for T cell therapy we are also developing new viral vectors (lentivirus and adenovirus) for efficient transfer of CAR transgenes to T lymphocytes and other hematologic cells. In addition, we are developing optimized protocols to expand the engineered T cells to make them resistant to oxidative stress and immunosuppressive factor.

Allogeneic DC vaccination

Patient-derived DCs modified with u-associated antigens have been evaluated as therapeutic cancer vaccines with some success. It has however become clear that *ex vivo*-modified DCs are short-lived when re-injected and do not migrate to draining lymph nodes. The therapeutic effect obtained from administration of *ex vivo*-modified DCs, with respect to functionality and maturation characteristics, appears to come from resident tissue (bystander) DCs that take up material from dying injected DCs and bring it to lymph nodes for antigen presentation to naïve T and B cells.

We therefore investigate if allogeneic DCs (DCs from a different individual) or a DC cell line can be used instead of patient-derived DCs. The logistic would be simplified and costs would be significantly reduced. Importantly, the HLA mismatch will most likely act as a strong adjuvant both for NK and T cells. We perform both efficacy studies and mechanistic studies in mice to evaluate which cell types are attracted and activated in response to allogeneic DCs. We use real-time intravital confocal microscopy imaging, in collaboration with Mia Phillipson, Uppsala University to study these events. We also investigate whether the therapeutic effect can be improved if the allogeneic DCs are transduced with an adenoviral vector secreting HP-NAP, IL-1b and other immune modulators.

Group members during 2015

Magnus Essand, professor, group leader
Alex Karlsson-Parra, adjunct professor, chief physician
Berith Nilsson, project leader
Minttu-Maria Martikainen, research technician

Justyna Leja-Jarblad, researcher
Di Yu, post doc
Victoria Hillerdal, postdoc
Miika Martikainen, post doc
Grammatiki Fotaki, PhD student
Chuan Jin, PhD student
Jing Ma, scholarship student
Mohanraj Ramachandran, PhD student
Tina Sarén, project degree student
Iliana Kerzeli, project degree student

Funding during 2015

Swedish Research Council, 1 000 kSEK
Swedish Cancer Society, 800 kSEK
Swedish Childhood Cancer Foundation, 500 kSEK
Immunicum AB, 950 kSEK
Victoory NET, 1 000 kSEK

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Diabetes Research

Olle Korsgren

Our research focuses on the cause of diabetes and on possibilities to prevent and cure the disease. The research has a broad multidisciplinary translational approach, which integrates genetics, bioinformatics, physiology, cell biology, clinical immunology, diabetology and transplantation research.

Diabetes mellitus is a lifelong, incapacitating disease affecting multiple organs. Estimates of worldwide prevalence suggest that 250 million patients have diabetes today and that this number by 2025 will increase by fifty per cent. In Sweden, at least 500,000 persons suffer from diabetes today. Diabetes and its complications impose an immense burden on the quality of life of patients and account for more than ten per cent of health care costs in Sweden.

Although type 2 diabetes accounts for most of the diabetes epidemic, type 1 diabetes (TID) is in Sweden the most common chronic disorder in children. More than two children per day are diagnosed with T1D, reaching more than 800 patients per year. In Finland one child out of 123 will be diagnosed with T1D before the age of 15 years. The figures are frightening and for unknown reasons the incidence of T1D has doubled during the past twenty years and continues to increase by four to six percent per year.

The aim of our research is to clarify the etiology of TID and to pave the way for development of new strategies for prevention and cure of TID.

The work is organised in five projects with the following objectives:

a) Unravel the etiology of TID.
b) Halt or prevent TID in newly diagnosed patients by transplanation of autologous mesenchymal stem cells.
c) Islet Imaging: Antibody-based proteomics for discovery and exploration of proteins expressed in pancreatic islets
d) Transplantation of isolated islets to cure patients with the most severe TID, experimental and clinical studies.
e) Induction of immunological tolerance: Regulatory T cells for treatment of transplantation induced immune reactions

Group members during 2015

Olle Korsgren, professor, group leader
Mahesh Kumar Anagandula, PhD student
David Berglund, researcher
Marcus Bergström, PhD student
Torsten Eich, PhD student
Maja Ericsson, research assistant
Karin Fonnaland, research engineer
Maria Hårdstedt, researcher
Sofie Ingvast, research engineer
Marie Karlsson, research engineer
Enida Kuric, research engineer
Marcus Lundberg, PhD student
Johan Olerud, researcher
Jonas Persson, researcher
Oskar Skog, research engineer
Per-Anton Stenwall, PhD student

Magnus Ståhle, PhD student
Anna-Maria Ullbors, adm. assistant
Anna Wiberg, PhD student

Dissertations during 2015

Magnus Ståhle, Technical challenges in human islet isolation. September 18, 2015.

Funding during 2015

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Diabetes Wellness, 1 000 kSEK
Swedish Research Council, 1 500 kSEK
Swedish Research Council, 952 kSEK (PI: Dept Med Sci)
Barndiabetesfonden, 233 kSEK
Exodiab, 8 680 kSEK
JDRF, 160 kUSD
EU FP7 (Pevnet), 311 k€
EU FP7 (HumEn), 127 k€
EU H2020 (Elastislet), 134 k€

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Immunotherapy for Cancer and Autoimmune Diseases

Angelica Loskog

Our research group develops new immunotherapies for cancer and autoimmune diseases. The immune system has an important role both in the development and control of these diseases and our research is based on the potential to affect the disease by modifying the immune response.

Tumor cells differ from normal cells both in appearance and growth pattern. They are therefore often recognized and killed by cells of the immune system. However, some tumor cells avoid recognition, for instance by producing immunosuppressive substances. These cells will continue to grow in an uncontrolled way, eventually causing cancer. We use gene technology to enhance anti-tumor immune reactions. For example, we are evaluating gene engineered T cells for the treatment of lymphoma and leukemia and immunostimulating gene therapy for the treatment of solid cancer such as melanoma and pancreatic cancer.

Multiple sclerosis (MS) is an autoimmune disease where the immune system attacks cells in the nervous system. We are investigating the immunological mechanisms of hematopoetic stem cell transplantation for MS patients.

Immunostimulatory gene therapy for cancer

The immune system has the capacity to destroy tumor cells by the same mechanisms that it clears viral infections. However, tumor cells require skills to turn off, or even kill, immune cells. We are investigating the role of different immune escape mechanisms and how they are affected by conventional or experimental treatment. By genetic engineering it is possible to shift the immunosuppressive milieu and/or to shield the effector immune cells from tumor-induced escape mechanisms. In this project the overall goal is to develop novel biological therapies for cancer focused on gene engineering cells and tumor tissues.

CD40 ligand (CD40L) is an immunostimulatory molecule that can be transferred to the tumor site by adenoviral vectors. CD40L production in the tumor area will enhance immune activation against the tumor resulting in tumor cell destruction, reduce the level of immunosuppressive molecules in the tumor area and drive Th1-mediated cytokine production. Moreover, stimulation of CD40 present on certain tumors such as those of epithelial origin will lead to tumor cell apoptosis which not only lead to decreased tumor mass but as well to increased uptake by antigen-presenting cells. We are evaluating the effects of AdCD40L gene therapy on tumors in both experimental models and in collaboration with other researchers at IGP as well as with Lokon Pharma AB and the Dept of Oncology at Uppsala University Hospital we are performing clinical Phase I/II trials on solid tumors currently with a focus on melanoma and pancreatic cancer.

T-cells are immune effector cells with high capacity to target and kill tumor cells. Adoptive transfer of *ex vivo*-cultured and expanded tumor-reactive T-cells has been investigated extensively. Due to the sensitivity of these cells to tumor-induced immunosuppression novel means are needed to enhance their survival and to restore their killing capacity. Lately, T-cells have been strengthened by gene technology prior to infusion into patients and multiple clinical trials are ongoing worldwide to test their safety and efficacy. In collaboration with Baylor College of Medicine, Houston, TX, Vecura at Karolinska University Hospital and Dept of Oncology at Uppsala University Hospital we have just initiated a clinical trial using CD19-targeting chimeric antigen receptor (CAR) T cells for lymphoma and leukemia and we are also developing novel improved gene technology vectors that are currently evaluated in preclinical models.

Development of novel therapies for multiple sclerosis (MS)

MS is an autoimmune disease of the central nervous system (CNS) in which the immune system attacks myelin-producing cells. The immune attack results in the destruction of the myelin sheet that covers nerves which leads to deteriorated function and may, in severe forms, cause paralysis. Most patients exhibit relapsing-remitting MS (RRMS) and these patients have shown possible to treat with autologous hematopoetic stem cell transfer (HSCT). Within this project we investigate the role of the immune system during different phases of the disease (relapse and remission) to determine how and why the immune cells are activated against myelin and why the normal tolerance mechanisms fail to prevent immune attacks during relapses. Patients subjected to HSCT stops to relapse and can even recover from previous symptoms to some extent. The major part of our current work is related to these patients and how HSCT has affected the immune system. This project is done in collaboration with the Dept of Neurology at Uppsala University Hospital. In experimental models we have investigated CNS-targeting immunosuppressive cells developed in our lab by genetic engineering. These cells target the CNS and locally suppress unwanted immune reactions without hampering peripheral control of infectious disease.

Group members during 2015

Angelica Loskog, professor (adj), group leader
Joachim Burman, post doc, specialist in neurology
Gustav Gammelgård, teaching assistant
Hannah Karlsson, researcher
Emma Eriksson, PhD student
Yoanna Milenova, research assistant
Gabriella Paul Wetterberg, engineer
Stina Söderlund, PhD student, resident in hematology
Ann-Charlotte Hellström, technical assistant
Jessica Wenthe, research assistant

Funding during 2015

Swedish Cancer Society, 800 kSEK
Swedish Childhood Cancer Foundation, 350 kSEK
AFA Insurance, 1 000 kSEK
Contract Research Lokon Pharma AB, 1 500 kSEK

Publications 2013-2015

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Blood Vessel Function after Transplantation

Peetra Magnusson

Disturbances in vascular function contribute to the development of several diseases and as a further consequence to human mortality. Diseases such as diabetes, heart failure and ischemia reperfusion injury share many of the same risk factors and consequential damage of the endothelium.

In replacement or regeneration of human cells, tissues or organs there is a need of functional revascularization. To improve recruitment of recipient's vessels to the new tissue utilization of biomaterials or supportive cells is an attractive strategy.

Our research focuses on the endothelium in health and disease. By using cutting edge techniques and our signature methods for endothelial cell interactions with the blood compartment, stem cells and heparin compounds we are investigating different possibilities to protect the endothelium in disease and to improve islet transplantation.

Ischemia reperfusion injury

A majority of kidney complications in diabetic patients lead to end-stage renal disease causing a need of kidney replacement. A challenge in organ transplantation is the great risk of ischemia-reperfusion injury occurring when the organ is connected to the vasculature of the recipient that may cause endothelial cell activation, triggering events leading to microvascular thrombosis and severe risk of graft failure.

Strategies to protect the vasculature upon transplantation are crucial. To be able to investigate the effects upon activated endothelial cells on a cellular and molecular level we are using our described blood endothelial cell chamber model where therapies with complement regulators/inhibitors will be investigated.

The strategy to protect the vasculature in transplantation is part of an EU FP7 supported project, DIREKT. The DIREKT project is coordinated from Uppsala by Prof. Bo Nilsson and the consortium has partners in Sweden, Norway, Denmark, The Netherlands, Greece, USA, Germany and Australia.

Heparin conjugate for vascular protection

In collaboration with Tomas Lorant (UU) and Corline Systems AB, Uppsala.

During a 2-year period the project "Heparin conjugate for vascular protection" will through funding from BIO-X/Vinnova develop the Corline Heparin Conjugate (CHC) into an *ex vivo* tool for repairing ischemia reperfusion injury (IRI) in kidneys prior to transplantation.

The aim of the project is to show that CHC will significantly reduce the vascular reperfusion injury for donated kidneys. The clinical therapy will use CHC to counteract the devastating effects of thrombosis that occurs upon reperfusion of the kidneys. Attenuating reperfusion injury will possibly improve function of the donated kidneys and reduce delayed graft function (DGF) after transplantation. Tasks related to market analysis, market plans and regulatory/toxicology will be addressed during the project period.

The project will fill an important gap by providing proof of concept (POC) data needed for submitting a clinical trial application to the Swedish Medical Product Agency (MPA).

Tissue bioengineering utilizing mesenchymal stromal cells

In collaboration with Katarina LeBlanc (Karolinska Institute), Olle Korsgren, Joey Lau, (UU) and the Science for Life laboratories

Mesenchymal stromal stem cells (MSC) are a heterogeneous population of stem cells that originates from the bone marrow and other tissues. Bone marrow derived MSC are currently used in the clinic in patients with graft *vs* host disease (GvHD) with promising results and are at present subjects for clinical trials for a variety of diseases.

We have via collaboration with the Karolinska Institute access to human MSC from healthy donors and are currently investigating their role in health and disease. It is well known that MSC migrate to inflamed tissues and we have observed that MSC are communicating with neighboring cells via organelle transfer. By using cutting edge techniques such as CyTOF, Seahorse, FlowSight, 2-photon microscopy, LightSheet and SPIM we are able to define the organelle transfer and the effect it has on the recipient cell. We have established a co-culture protocol of MSC and endothelial cells that allow investigations of cellular mechanisms and function.

In the process of revascularization upon transplantation MSC can support endothelial cells by the production of growth factors and matrix proteins. MSC also produce proteases enabling vessels to migrate into the surrounding tissue during angiogenesis. We have a model system of combining MSC with islets of Langerhans investigating their cellular contributions to the graft. Furthermore, we are investigating their potential in supporting a vascularized site pre transplantation by utilizing biomaterials and surface treatments.

Group members during 2015

Peetra Magnusson, researcher, group leader
Maya Arvidsson, degree project student
Johan Brännström, research engineer
Fredrik Carlsson, researcher
Fredrik Edin, PhD student
Joakim Folkesson, degree project student
Moa Fransson, post doc
Sofia Nordling, PhD student
Linus Sanner, student
Amir Sedigh, physician

Funding during 2015

Diabetes Wellness, 250 kSEK
Barndiabetesfonden, 155 kSEK
Swedish Heart-Lung Foundation, 138 kSEK
Vleugels Fond, 390 kSEK

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Thromboinflammation in Therapeutic Medicine

Bo Nilsson

The cascade systems of the blood consist of the complement, the contact, the coagulation and the fibrinolysis systems. In particular the complement system, but also the other systems, are part of the innate immune system. The primary function of the complement system is to act as a purging system of the body to remove foreign substances including microorganisms, apoptotic cell debris, immune complexes and foreign bodies/materials. The primary function of the fibrinolysis, the coagulation and the contact systems is in hemostasis. However, all three systems are also engaged in inflammation.

Physiologically, thromboinflammation is an initiator of the healing and repair process of the body and is triggered by the humoral innate immune system, which primarily consists of the cascade systems of the blood. These subsequently activate leukocytes, platelets and endothelial cells, finally resulting in thrombotic and inflammatory reactions. Thromboinflammation is also an important pathophysiological process involved is several clinical conditions and treatments:

1. Cell and cell cluster transplantation and therapies.
2. Whole organ transplantation
3. Thrombotic events such as cardiac infarction, stroke and other cardiovascular conditions
4. Rheumatic conditions (scleroderma, SLE, antiphospholipid syndrome).
5. Pharmacological delivery systems e.g. lipid miscelles, polymers, virus vectors etc.
6. Treatments with biomaterials implants (joint replacements, scaffolds for tissue engineering etc), extracorporeal treatments (hemodialysis, cardiopulmonary bypass).

Cross-talk between the cascade systems and activated platelets

Osama Hamad, Huda Kozarcanin, Kristina Nilsson Ekdahl, Bo Nilsson

Platelet activation during thrombotic events is closely associated with complement and contact system activation, which in turn leads to inflammation. Chondroitin sulfate A (CS-A), released from alpha granules during platelet activation, is a potent mediator of cross-talk between platelets and the complement system. Under physiological conditions, no complement activation seems to occur on the activated platelet surface, but C3 in the form of C3(H2O) is bound to the surfaces of activated platelets. C3(H2O) is a non-proteolytically cleaved but activated form of C3, with C3b-like properties. Platelet-bound C3(H2O) acts as a ligand for leukocyte CD35 and CD11b/CD18, enabling platelet-leukocyte interactions.

Furthermore, we have shown that activated platelets and fibrin elicit activation of the lectin pathway enzymes, MASP-1 and -2 without complement activation. The MASP proteases thereby represent a crossover between the complement and coagulation. Thus, in addition to their traditional role as initiators of secondary hemostasis, platelets also act as mediator and regulator of inflammation in thrombotic events. This project is supported by the Swedish Medical Research Council, VR.

Disarming the intravascular innate immune response to improve treatment modalities for chronic kidney disease

Sana Asif, Karin Fromell, Yuji Teramura, Andreea Barbu, Kristina Nilsson Ekdahl, Bo Nilsson

Chronic kidney disease is world wide a major cause of end-stage renal disease (ESRD). 800.000 patients in Europe and in the US, respectively, require long-term treatment initially with peritoneal dialysis, followed by hemodialysis and kidney transplantation. Each ESRD

patient on hemodialysis costs ≈€40000 to €80000 per year, has extremely poor quality of life and an average life expectancy of only 4 years. Kidney transplantation totally changes life for ESRD patients who can then return to normal life, but this treatment is hampered by the low number of available kidney grafts. All these treatments are, however, associated with adverse reactions that cause damaging thromboinflammation, triggered by the intravascular innate immune system, which may lead to poor results and non-function.

The overall aim of this project is to clarify the innate immune mechanisms that cause thromboinflammation and identify nature's own specific control points of regulation in these adverse reactions. By applying these concepts of regulation in hemodialysis and kidney transplantation, we intend to significantly improve the quality of hemodialysis devices and kidney grafts. We envisage to 1) convey a novel soluble complement inhibitor to the clinical stage via phase 1/2a clinical studies, 2) create of nano-profiled surfaces with low activating properties and 3) generate easy-to-apply one step-coatings for treatment of biomaterials (hemodialysis) and endothelial cell surfaces (kidney grafts) that will significantly improve the treatment modalities of ESRD. We expect that these advances will result in extended periods during which hemodialysis can be applied to patients and that the quality of life will improve. In kidney transplantation attenuation of innate immune reactions is anticipated to protect the grafts against damage thereby making a larger number of kidneys accessible for transplantation. The novel techniques are also likely to be applicable on other types of implantations, extracorporeal treatments and transplantations and in the future to be used in xenotransplantation and stem cell therapies. This project is part of the FP7 grant DIREKT coordinated by our group.

Thromboinflammation induced by nanoparticles

Padideh Davoodpour, Jaan Hong, Bo Nilsson, Kristina Nilsson Ekdahl

Nanoparticles (NP) and nanostructured materials are used in a growing number of applications and their use is expected to increase dramatically in the future. We have found that NP of different origin induce thromboinflammation, and our aim is to apply the technology that we developed for elucidating the biocompatibility of biomaterials in contact with blood, to characterize the biological responses and toxicity of NP in contact with tissue fluid / blood plasma / whole blood. We have applied this technology to investigate TiO2 NPs. These particles are widely used and applied in a number of applications e.g. sun protection, white paint and toothpaste etc. Our investigations have revealed that they are highly thrombogenic, despite that they have been considered to be mostly inert. The project will help to clarify the mechanisms of toxicity of NPs, and help to develop techniques for evaluating the toxicity of present and future NP materials that are disseminated in the environment. This project is supported by AFA.

Coatings of liposomes in order to avoid innate immune recognition

Claudia Dührkop, Bo Nilsson, Kristina Nilsson Ekdahl

Drug delivery by liposomes is a technique to contain and neutralize toxic drugs, e.g. various chemotherapies, in order to avoid release of the drug to off-target cells. Liposomes injected into the blood are, however, recognized by the innate immune system, leading to accelerated removal of the particles and to adverse reactions. Attempt to conceal the surface with polyethyleneglycol (PEG) have been partially successful, but also this coat has been shown to be recognized by the innate immune system. The so-called accelerated blood clearance (ABC) phenomenon has been suggested to be triggered by natural IgM antibodies. In this project, which is supported by the FP7 project DECENT AID, we attempt to find alternative coatings to avoid innate immune recognition and ABC.

Coatings of liposomes in order to avoid innate immune recognition

Jaan Hong, Bo Nilsson, Kristina Ekdahl

Development of biomaterials intended for applications in tissue engineering is time-consuming and costly, due to design of the material and repeated testing in animal models. Therefore, there is a need to find screening techniques that at an early stage can be used to predict the biocompatibility of the material. One of the major properties of a biomaterial that determines the fate of the implant is the recognition by the innate immune system. We have developed two different tentative screening techniques that are applicable for this purpose. The first one employs the adsorbed protein profile after exposing the material to blood plasma. We have demonstrated that the proportion of complement and coagulation protein profiles, are closely correlated with the biological response. The second technique is a migration assay that allows blood cells to migrate through a membrane in response activation products generated by the biomaterial in contact with blood plasma. Both assays are at present under evaluation in animal models. This project is supported by FP7 Project BIODESIGN.

Group members during 2015

Bo Nilsson, professor, group leader
Sana Asif, PhD student
Andreea Barbu, researcher
Padideh Davoodpour, researcher
Karin Fromell, researcher
Elisabet Gustafson, MD, PhD student
Osama Hamad, researcher
Jaan Hong, researcher
Huda Kozarcanin, PhD student
Susanne Lindblom, research engineer
Kristina Nilsson Ekdahl, visiting professor
Lillemor Stenbeck-Funke, research engineer
Elisabeth Wijkström, research engineer

Funding during 2015

Swedish Research Council, 1 000 kSEK
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EU FP7 (Decent Aid), 180 k€
EU FP7 (Direkt), 480 k€

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Immune Complexes in Rheumatic Diseases

Johan Rönnelid

Our research focuses on the functional and prognostic impact of immune complexes and immune complex-associated autoantibodies in rheumatic diseases and chronic infections. We study immune complex (IC)-mediated mechanisms in chronic rheumatic diseases, primarily rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and chronic infections like *Leishmania donovani*. We also study how IC and IC-associated autoantibodies act as prognostic markers for future disease development.

Our research aims to make IC more central in the etiologic perception of chronic diseases from a modernized functional approach. This can lead to definition of new IC-dependent disease phenotypes, as has been the case in RA, as well as to phenotype-based therapies in autoimmune diseases with IC-driven pathology.

Characterization of immune complexes

Vivek Anand Manivel, Azita Sohrabian, Amir Elshafie, Linda Mathsson, Mohammed Mullazehi, Sahwa Elbagir, Johan Rönnelid

The project relies on close collaboration between basic immunological and clinical research, mostly within rheumatology. The basic research concerns characterization of IC-induced immune/inflammatory reactions and development of new techniques to measure effects of IC and IC-associated autoantibodies. The measurement outcomes are then related to the clinical situation for the individual patients at the time of sampling (pathogenetic issues) or later (prognostic issues).

Our present interest is to evaluate the prognostic impact of autoantibody levels within IC. We have shown that these levels show changes over time that fundamentally differ from the changes in serum. We are currently investigating whether such changes have prognostic impact by analysing serum and IC levels over time in SLE patients treated with antibodies depleting all B cells (Rituximab) and neutralizing B-cell activating factor (Belimumab).

The role of IC in disease

Vivek Anand Manivel, Azita Sohrabian, Amir Elshafie, Linda Mathsson, Mohammed Mullazehi, Johan Rönnelid

At the clinical level we investigate the importance of IC-triggered mechanisms for the development and maintenance of disease activity in RA, SLE and chronic infections. One of our main interests is currently to describe in detail the group of RA patients with high levels of circulating autoantibodies reacting with collagen type II in joint cartilage. We have shown that these antibodies, which show the highest levels very early (at the time of RA diagnosis) are found in patients which also have maximum inflammation and joint destruction at this early time point.

With two *in vitro* models reflecting anti-collagen containing IC in the joints, we have shown that these IC induce the production of inflammation-promoting and joint-degrading substances. Thereby we have explained the link between the early appearance of anti-collagen antibodies and the simultaneously appearing inflammation and joint destruction in anti-collagen antibody positive RA patients.

We purify IC from blood or inflamed joints, whereupon these IC are used to stimulate cells *in vitro*. In other *in vitro* systems we create artificial IC with human components, and use these IC to stimulate different cell types. In these experiments we aim to mimic immune reactions that take place in specific target organs in patients, e.g. RA cartilage or in the soft

tissues in close vicinity to bone/cartilage erosion in RA joints. This work is done in close collaboration with researchers from many rheumatology centers in Sweden, Holland, United Kingdom, USA and Sudan, as well as tropical medicine specialists in Sudan.

We believe that a greater functional understanding of IC-mediated mechanisms can lead to new principles of treatment in IC-associated diseases like RA, SLE and chronic infections. Such knowledge will also lead to better understanding and distinguishing of pathogenetically separate subgroups of patients in traditional criterion-based diseases like RA and SLE. Thereby it will be possible to treat each phenotypical patient subgroup in an individually and biologically adequate way.

Comparative studies of rheumatic diseases in Sweden and Sudan

Amir Elshafie, Sahwa Elbagir, Johan Rönnelid

Little is today known about the natural history of rheumatoid arthritis in third world countries, and nothig has been published from Sudan. In the first part of the project we investigate Sudanese RA patient and preliminary data show very high disease activity and severe joint destructions.

The world's highest rates of stillbirths are found in sub-Saharan Africa. The anti-phospholipid syndrome (APS) is characterized by thromboses and severe pregnancy complications. APS is associated with anti-phospholipid antibodies, and often related to systemic lupus erythematosus (SLE) a disease with a very strong female preponderance and increased pregnancy risk. The second part of the project we investigate Sudanese pregnancies and SLE patients in an APS context.

In both the Sudanese projects we compare clinical manifestations, autoantibody profiles, and genes (HLA and Genome Wide Association Studies).

Group members during 2015

Johan Rönnelid, adjunct professor, senior consultant in clinical immunology, group leader
Azita Sohrabian, research engineer/PhD student
Sahwa Elbagir, PhD student
Vivek Anand Manivel, PhD student
Barbro Persson, physician
Amir Elshafie, post doc
Linda Mathsson, affiliated researcher
Mohammed Mullazehi, affiliated researcher
Chiara Beretta, project student

Funding during 2015

Swedish Research Council, 700 kSEK
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Swedish Rheumatism Association, 175 kSEK
King Gustav V:th 80-year foundation, 100 kSEK

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Immunotherapy of Cancer

Thomas Tötterman, Sara Mangsbo

Growing tumors have the capacity to counteract the attack and control of the immune system by creating an immunosuppressive milieu. This is the result of recruitment of several types of immunosuppressive cells and their cytokines.

We have initiated a series of novel locally applied immunotherapies in which we aim to revert this negative milieu. Therapies include the use of Adenovectors expressing CD40L, monoclonal antibodies and Toll-like receptor agonists. We have pioneered local AdCD40L immunotherapy of bladder cancer and melanoma in man and melanoma in the dog. Our adoptive T cell therapy trial in human melanoma was one of the first. Our group were also involved in the pre-clinical validation of ADC-1013 (Mangsbo et al. Clin Canc Res 2015), a CD40 specific antibody developed by Alligator Bioscience and currently licensed to J&J. In addition we are currently developing a novel therapeutic long peptide vaccine, delivered to dendritic cells by antibodies, for the treatment of prostate cancer. The project is financed by BIO-X/Vinnova and is a collaboration effort between Uppsala University, Leiden University Medical Center and Immuneed AB.

Immune stimulating gene therapy

Over the past several years, we have developed and characterized Adenoviral vectors expressing immunostimulatory genes in several tumor models, with special focus on bladder cancer. Injection of AdCD40L directly into the tumor area effects tumor regression and specific immunity. A first-in-man clinical phase I/IIa study utilizing this vector in aggressive bladder cancer has been published. Several patients experienced tumor regression or disappearence, with minimal side effects. We have in collaboration with SLU (Swedish Agricultural University) treated 19 dogs with aggressive malignant melanoma, again with very encouraging results. We are currently pursuing a clinical trial with our AdCD40L therapy on melanoma patients. The therapy is given with or without low dose cyclophosphamide and the latter is applied as means to inhibit the function T regulatory cells thereby aiding anti-tumor responses. We have also, together with professor Magnus Essand, validated a second generation vector for gene therapy that can target a wider range of cells allowing us to modulate the whole tumor micro environment including antigen-presenting cells (Liljenfeldt et al JIT 2014).

Adjuvant therapies in combination a block of inhibitory receptors to target tumors

Immune activation can be hampered by two major immune checkpoint regulators (CTLA-4 and PD-1). In order to ensure proper and sustained T cell activation one can use antibodies that block these two receptors. We have combined the synthetic DNA sequences; CpG ODNs (described in the previous project) with CTLA-4 or PD-1 blockade to examine if the combination strategy could improve therapy.

Single and combination strategies were assessed in an experimental bladder cancer model. CTLA-4 blockade alone prolonged survival of mice. When anti-CTLA-4 or anti-PD-1 antibodies were combined with CpG, survival was enhanced and elevated levels of activated T cells were found in treated mice. We believe that this strategy can be used to further improve on immunotherapy for patients with aggressive bladder cancer or other solid tumors. Together with collaborators in the US we are now actively investigating how to optimize check-point therapy in bladder cancer, focusing on local anti-CTLA-4 therapy alone, or in combination with systemic anti-PD1.

Cancer vaccines

For the last years we have pursued a track of research aiming to improve T cell priming/activation by facilitating the delivery of synthetic long peptides (SLPs) into DCs via Fc receptors (FcR) The SLPs are overlapping ~20-30 long amino acid sequences spanning tumor or pathogen related antigens (Ags) and can be used to trigger T cell responses in conjunction with adjuvants. SLPs have the advantage, over short single peptides, to span a whole tumor associated protein. They include a plethora of CD4 and CD8 T cell epitopes for various HLA alleles. Importantly, they require processing by antigen-presenting cells (APCs) and will therefore not directly interact with MHC class I on non APCs, thus lowering the risk of anergy induction.

SLPs have successfully been assessed by our collaborators in Leiden in a clinical trial for high-grade vulvar intraepithelial neoplasia using long peptides spanning the E6 and E7 oncoproteins but the work demonstrate that improvements are needed to cure larger lesions. Our work to improve SLP vaccination has led to the discovery that a B cell epitope (a hapten/Ag), when coupled to SLPs, can facilitate Ag-SLP uptake. The idea is that circulating antibodies (Abs) will bind the hapten and immune complexes will form that can subsequently interact with Fc receptors which will lead to Ag-SLP uptake, processing and presentation to T cells. The subsequent T cell response will be improved as the DCs are loaded with significantly more Ag-SLP due to immune complex mediated uptake. Additionally DCs are activated by the FcR interaction, enabling upregulation of CD80/CD86 as well as cytokines, crucially important for optimal T cell activation (Schematic illustration in Figure 1).

We are currently investigating this novel vaccine in a human blood loop system to establish how the immune complexes behave in the presence of intact human blood components.

Via funding from Bio-X (Vinnova) we are now preparing a clinical grade batch of a prostate cancer vaccine based on long peptides with the aim to progress to a clinical trial in Q4 2017. The project also includes proof-of-concept as well as toxicity studies and is performed with LUMC and Immuneed AB as partners.

Myeloid cells in the tumor micro environment

We are collaborating with both industrial and academic partners in the TIMCC network (EU Marie Curie ITN grant to Dr Mangsbo and associate professor Dimberg). Our previous data in this area demonstrate that we can affect myeloid cells by our well know AdCD40L therapy. Herein we are further exploring how this recruitment and modulation of myeloid cells occur in response to immunotherapy and how the vasculature can affect this. This is an exciting new project that will continue until the end of 2016.

Group members during 2015

Thomas Tötterman, professor, group leader
Sara Mangsbo, researcher, assisting group leader
Erika Fletcher, PhD student
Ann-Charlotte Hellström, technician
Gabriella Paul-Wetterberg, lab engineer
Wictor Gustafsson, research assistant
Justyna Leja-Jarblad, researcher
Luuk van Hooren, PhD student
Frida Lindqvist, student

Funding during 2015

Thomas Tötterman
Swedish Cancer Society, 850 kSEK
ALF, 290 kSEK

Sara Mangsbo
Vinnova/BIO-X, 1 000 kSEK
Göran Gustafsson Stiftelse, 500 kSEK

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Experimental and Clinical Oncology

The overall goal for the research in this programme is to gain insight into the complex molecular mechanisms underlying tumour evolution, and to identify novel prognostic and/or predictive markers and treatment targets for intervention using true precision medicine in cancer. In addition, the consequences of the disease and treatment for the physical, mental and social dimensions of patients' wellbeing are investigated.

The research programme includes experimental projects that are performed in the laboratory. Using modern molecular and cellular methods, we study patient derived tumour cells and relevant models for the tumour *in vitro* and *in vivo* to identify essential pathways that may cause cancer or that will affect disease progression. We also want to find novel biomarkers that can be used for improved diagnostics and prognostication, or that may function as targets for new treatment strategies.

Our clinical research programme includes thorough evaluation of the effect of different treatment strategies to find ways to determine which therapy is most efficient for the individual patients. New treatment modalities including immunotherapy are also studied. With the aim to improve immediate patient care, we also study the effects of interventions to diminish treatment toxicity and improve health related quality of life.

Tumour biology and clinical studies of lymphomas and clinical studies of prostate cancer and ovarian cancer

Gunilla Enblad

We are studying different types of lymphomas i.e. diffuse large B-cell lymphoma, Hodgkin lymphoma and mantle cell lymphoma. Our goal is to increase the knowledge about the biology behind the diseases and how afflicted patients can be treated in the best way. We also want to contribute to the development of new, improved treatment strategies.

Lymphomas are a group of tumours that originate from the lymph system and where white blood cells grow in an uncontrolled way. There are several types of lymphoma that can be more or less aggressive. Diffuse large B-cell lymphoma is one of the most common types of lymphoma. It is an aggressive type and patients that are not treated have a short survival. Hodgkin lymphoma is less common but often affect younger patients and have a good prognosis but late side effects of treatment is a threat to the patient's health. Mantle cell lymphoma is a very aggressive lymphoma with a poor prognosis.

Biology of diffuse large B-cell lymphoma

Gunilla Enblad, Mattias Berglund, Gustaf Hedström, Charlott Mörth, Amal Abu Sabaa, Alex Gholiha, Antonis Valachis

In our research we study the biological background for the origin and growth of the tumour. We have previous observed that patients with an autoimmune disease such as rheumatoid arthritis have a higher risk of developing diffuse large B-cell lymphoma. There are also

gender and age differences linked to disease prognosis. We are using the U-CAN material to study tumor material and serum and plasma proteins to elucidate the biology of the disease. The project is a collaboration within Uppsala University with Rose-Marie Amini, Eva Baecklund, Christer Sundström, Maysaa Aslani, Larry Mansouri, Richard Rosenquist.

Development of new therapies

Gunilla Enblad, Hans Hagberg, Maryam Delforoush, Alex Gholiha

Another aim is to develop new therapies for patients who do not respond to the standard treatments used today. We are for instance working with a new strategy where genetically modified T cells, a type of white blood cell, are used to eradicate the tumour cells. We also analyse the effect of new drugs in animal models, with the objective to use the most promising drug candidate in clinical studies. The project is performed in collaboration with Angelica Loskog, Magnus Essand and Joachim Gullbo.

Clinical and biological studies of Hodgkin lymphoma

Daniel Molin, Gunilla Enblad, Ingrid Glimelius, Peter Hollander, Ingemar Lagerlöf, Ninja Övergaard, Ulla Martinsson

The aim is to increase the knowledge of the interaction between the tumour cells and the microenvironment in Hodgkin lymphoma and how this knowledge can be used to develop new treatments. Furthermore our aim is to study FDG-PET in relation to the microenvironment and to perform clinical studies on patients with Hodgkin lymphoma. Lastly, our aim is to study late effects of the treatment and how they can be avoided. The project is performed in collaboration with Rose-Marie Amini, Gustaf Ljungman and Annika Englund.

Clinical and biological studies of Mantle cell lymphoma

Ingrid Glimelius, Anna Laurell

The aim of this project is to introduce and study new treatments in mantle cell lymphoma. Furthermore, we aim to study the microenvironment in relation to clinical outcome and also if these patients have any significant late effects of the treatment.

Clinical and biological studies of prostate cancer

Silvia Johansson, Lennart Åström, David Kudrén, Gunilla Enblad

The projects involve different aspects of radiotherapy for patients with prostate cancer and how the effects can be measured and described. Patients with localised prostate cancer treated with proton beam therapy or brachytherapy are evaluated. The biology of prostate cancer after a short course of radiotherapy is studied and the new treatment with Radium223 is studied and evaluated.

Clinical and biological studies of ovarian cancer

Ingrid Glimelius, Camilla Sköld, Gunilla Enblad

This project aims to study the risk of ovarian cancer in relation to pregnancy parameters. Furthermore, we aim to use the U-CAN material for studies of the tumour biology and biomarkers in relation to prognosis.

Group members during 2015

Gunilla Enblad, professor, group leader
Amal Abu Sabaa, MS PhD student
Mattias Berglund, researcher
Maryam Delforoush, PhD student
Alex Gholiha, MD PhD student
Ingrid Glimelius, MD ass professor
Hans Hagberg, MD ass professor
Gustaf Hedström, MD PhD
Peter Hollander, PhD student
David Kudrén, PhD student
Silvia Johansson, MD ass professor
Ingemar Lagerlöf, PhD student
Anna Laurell, MD PhD senior consultant
Ulla Martinsson, MD Ph D senior consultant
Daniel Molin, MD ass professor
Charlott Mörth, MD PhD student
Camilla Sköld, MD PhD student
Antonis Valachis, MD PhD senior consultant
Lennart Åström, MD PhD student
Ninja Övergaard, MD PhD student

Funding during 2015

Swedish Cancer Society, 600 kSEK (project grant), 150 kSEK (planning grant)
Swedish Childhood Cancer Foundation, 1 781 kSEK (to U. Martinsson)
Stiftelsen Onkologiska klinikens i Uppsala forskningsfond, 200 kSEK (G. Enblad), 920 kSEK (D. Molin), 258 kSEK (I. Glimelius)
ALF, 515 kSEK
Lions Cancer Research Foundation, 150 kSEK
Selanders Stiftelse, 100 kSEK

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Colorectal Cancer and Malignant Lymphoma

Bengt Glimelius

Our studies on colorectal cancer and malignant lymphoma aim to increase the knowledge about the diseases, develop new therapies and improve patient care. The overall goal is to improve the outcome for these common cancer types.

Our projects focus on clinically relevant aspects, from diagnosis and staging of primary disease, to care of the patients close to the end of life. We have driven a large number of projects, each with its own specific research aims. These include both studies to explore population-based quality registers, and prospective studies from phase I studies exploring new treatment concepts to large randomised phase III trials involving many centres in many countries.

Combining radiotherapy, chemotherapy and surgery to improve patient outcome

Bengt Glimelius, Calin Radu, Peter Nygren et al.

In collaboration with Helgi Birgisson (UU)

Preoperative radiotherapy has an established role in the treatment of many patients with primary rectal cancer, but it is presently still not possible to accurately identify those in the greatest need for the additional therapy. Other problems relate to the best timing of the surgery to the radiotherapy and integration of chemotherapy and newer target drugs. These are studied in several on-going trials, e.g. in the Stockholm III study where patient inclusion was recently completed.

A large randomised trial in locally advanced rectal cancer, testing the value of neo-adjuvant chemotherapy, is ongoing (Rapido). Collection of biologic material is done so that the tumours and patients best suited for a particular treatment can be identified. Discussion about a new large randomised trial is about to be finished.

Since we have run some of the largest radiotherapy trials in rectal cancer, we will further explore the risks of late-late toxicity such as anal and sexual function and overall quality-of-life in relation to radiation burden and patient characteristics. Studies about secondary malignancies, up to 25 years after radiotherapy, are soon completed.

Long-term follow-up of a 2 200 patient-large study evaluating the value of adjuvant chemotherapy in colorectal cancer is ongoing. The main aims are to evaluate factors predictive of recurrence and response to 5FU-based treatment.

Treatment of metastatic colorectal cancer

Bengt Glimelius, Åke Berglund, Peter Nygren

In metastatic disease, even if substantial improvements have been achieved, most patients will die from the disease. Several drugs, both conventional cytostatics and novel targeted drugs have activity, and are given in different combinations and lines. The best treatment strategy is not always known.

We have completed the Nordic VII trial comparing a combination of cytostatics without or with an EGFR-inhibitor (cetuximab). In a third arm, planned breaks were studied. Tissue blocks, serum and plasma and cells for DNA preparation have been collected and are analysed for identification of predictors of therapy response.

Studies with the aim of early prediction of response using functional imaging, tumour markers and patient-reported outcomes are on-going in a previous study, Nordic VI, also including more than 550 patients. A randomised study in the conversion situation, Nordic VIII is ongoing, and a study in elderly patients, Nordic IX has recently started.

In an effort together with one centre in Denmark and one in Norway, we identified every individual with metastatic colorectal cancer during a three-year period, with the possibilities to explore an unselected population. An early finding was that trial patients are far from representative of the general population. This appears also to relate to the presence of specific molecular events, like the presence of BRAF-mutations and loss of CDX2. These mutations/changes appear much more common in this unselected cohort than in clinical trials. A new similar collection is about to be lounged.

U-CAN material is used to identify colorectal cancer markers

Bengt Glimelius, Erik Osterman

In collaboration with many researchers at IGP and in Umeå

Colorectal cancer is one of the diagnoses in the U-CAN project, a collaborative project jointly between Uppsala and Umeå universities. The aim is to prospectively collect clinical information and biological material from diagnosis and during follow-up for research.

In collaboration with other research groups we will use the banked colorectal cancer material to study molecular markers detected in serum or plasma for the ability to early detect response and disease progression during neo-adjuvant, adjuvant and palliative chemotherapy and radiochemotherapy.

Cancer survivorship

Bengt Glimelius, Birgitta Johansson, Annika Thalén-Lindström

In collaboration with Lena Ring, Åsa Kettis (UU)

Parallel to the clinical studies in especially gastrointestinal cancer, we have continuously developed supportive care activities that focus on specific symptoms or general problems of psychological, social and existential matter. We have also explored the quality of life of the patients and the importance of socioeconomic status for treatment and outcome.

Our projects that aim to improve patient care are:

- Randomised studies to evaluate the value of different psychosocial care activities, including cognitive behavioural therapy (CBT). The aim is to identify better instruments to reliably predict patients who might be in need of early interventional therapy.
- A project comparing two quality-of-life instruments, the traditional EORTC QLQ-C30 and the individual quality-of-life instrument, SEIQoL.
- Many treatments are toxic and associated with both acute and late effects. In the completed Stockholm III study and in the ongoing RAPIDO-trial we study the negative long-term effects of radiation.
- Health care in Sweden aims to be equal to everyone but research has shown that this is not always the case. In one project we use quantitative and qualitative studies to explore the reasons for the discrepancies and activities to prevent them.

Aetiology of malignant lymphomas – the SCALE study

Bengt Glimelius, Ingrid Glimelius, Daniel Molin, Gunilla Enblad et al.

In collaboration with Karin Ekström-Smedby et al. (KI)

During the last decades there has been a marked increase in malignant lymphomas, although it has levelled off during the most recent years. In order to better understand the reasons behind malignant lymphomas and particularly the increase, we have performed a large population-based case control study in Sweden and Denmark. The participation rate was very high, with in total 3 740 cases and 3 187 controls included, and the studies have provided valuable information about risk factors for malignant lymphoma.

The SCALE study is the largest and most complete study in the international collaboration InterLymph, which also allows for analyses of very rare malignant lymphoma and the link between gene variants and incidence. Several studies focus on Hodgkin's lymphoma where the correlation between environmental factors, genetic characteristics, tumour cells and surrounding normal tissues is also studied.

Group members during 2015

Bengt Glimelius, professor, group leader
Åke Berglund, consultant
Nina Cavalli-Björkman, consultant
Inger Hjertström Östh, administrative assistant
Calin Radu, consultant

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Characterization of Novel Regulators of Blood Vessel Formation

Mats Hellström

In recent years scientists have clarified how important the formation of new blood vessels is in various diseases. Despite this fact, there is still a lack of knowledge about the signalling pathways that regulate blood vessel formation and only a few blood vessel-specific drugs have been developed.

Our research focuses on finding signalling components that are specific for endothelial cells, the cells that line the inner walls of blood vessels. We have identified several possible candidates and one of these, called paladin, we have analysed in more detail. We have shown that mice that lack paladin have altered blood vessels in the retina, and we are studying the role of paladin in tumour development. Paladin belongs to a group of proteins that are commonly involved in cell signalling. We hope that our results will contribute to an increased understanding of signalling during the formation of new blood vessels in tumours.

Characterization of Novel Regulators of blood vessel formation

Isabel Egana, Hiroshi Kaito, Anja Nitzsche, Chiara Testini

Although the importance of angiogenesis in pathological conditions is well established few blood vessel-specific drug targets have been identified and information is still limited about endothelial-specific molecular pathways. Hence, there is a great need to better characterize the process in order to provide new ideas for improved and novel therapies.

In the search for endothelial-specific regulators we have used several approaches, including expression profiling of mouse vasculature and other mouse tissues, zebrafish gene knock down, and screening of drug-like compounds in human cellular assays. This led to the identification of several new regulators of angiogenesis, including kiaa1274/x99384/Pald1 (or Paladin), a putative cytoplasmic phosphatase. Paladin is one of the first examples of a cytoplasmic, potential phosphatase with an endothelial-specific expression.

We have generated a mouse knock-out for *Paladin* with a functional β-galactosidase reporter, verifying endothelial specificity in many mouse tissues. We have characterized the expression pattern of mouse and human Paladin during development and in cancer tissue. Paladin is preferentially expressed in the vasculature and shows a dynamic expression pattern changing from expression in capillaries and veins during development, to vascular smooth muscle cells in arteries in the adult organs. The knock-out mice are viable and fertile. Our preliminary data show that Paladin knock-outs display increased vascular density in the postnatal retina. We plan to further study vascularization of normal tissues as well as tumors. We will also perform comprehensive biochemical and signal transduction analyses *in vitro*, including over-expression and siRNA knock down of *Pald1*.

Kinases belong to an important drug target class in oncology, which strongly suggests that our studies on *Pald1* will contribute to the understanding of kinase/phosphatase signaling in general and angiogenesis/tumor angiogenesis in particular.

Group members during 2015

Mats Hellström, researcher, group leader
Isabel Egaña, post doc
Hiroshi Kaito, post doc
Anja Nitzsche, PhD student
Chiara Testini, PhD student

Funding during 2015

Swedish Cancer Society 800 kSEK

Publications 2013-2015

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Molecular Characterization of Acute Leukemia

Linda Holmfeldt

Acute leukemia is a blood disorder that is diagnosed in about 400 Swedes annually. Despite the best possible treatment with the drugs available today, a large fraction of the patients do not respond to the treatment or experience a relapse after an initial response. It is not possible to increase the survival by intensifying the treatment, since the cancer medicines available today are toxic themselves. Thus, to find new, more efficient treatment alternatives with fewer side effects, more knowledge about the origin and growth of the cancer cell is needed.

Our research aims to increase the understanding of why many patients do not respond to treatment or suffer from a relapse of the disease. We also want to identify changes in the tumor cells that can be used to develop more efficient treatment alternatives for high-risk leukemia, that today is associated with a very poor outcome.

What are the underlying causes of initiation and progression of acute myeloid leukemia?

Svea Stratmann, Henrik Steffen

To identify which alterations that favour primary treatment failure and/or the outgrowth of resistant relapse clones, we perform unbiased multilevel analyses comparing newly diagnosed and relapse AML specimens.

Complementary high-resolution techniques are used to identify any alterations that may explain treatment failure or the onset of relapse. Techniques included are, amongst others, whole genome and/or exome sequencing, RNA sequencing and various studies of the epigenome. We also study the proteome of the leukemic cells, comparing these to corresponding normal cells. By employing systems biology approaches, data generated from the above mentioned analyses are integrated to generate hypotheses that could explain tumour progression.

To complement the exploratory studies, we functionally evaluate the hypotheses generated using a combination of cellular studies and *in vivo* modelling. Finally, evaluation of novel therapeutic alternatives for AML is performed.

What are the downstream consequences of aberrant epigenetic regulators in leukemia?

Ren Sun, Henrik Steffen

My previous studies of pediatric high-risk and relapsed acute lymphoblastic leukemia identified a high frequency of alterations of epigenetic modifiers. Among these, alterations in the histone H3K27 methyltransferase Polycomb repressive complex 2 (PRC2) stand out, especially the catalytic subunit EZH2, which has been shown to act as both an oncoprotein and tumor suppressor in different types of malignancies. This suggests that perturbation in epigenetic regulation facilitates a reduced response to therapy and/or the onset of relapse.

We want to answer the question whether specific alterations in epigenetic regulators cause i) stochastic changes at the epigenetic level, or ii) specific and recurrently found epigenetic changes of genes that favour tumorigenesis.

One of the aims in our lab is thus to interrogate the downstream consequences of aberrant PRC2 on leukemogenesis. The approach we take includes everything from biochemical enzymatic assays utilizing purified protein complexes, through analyses at the cellular level to *in vivo* modelling, followed by epigenomic and transcriptomic analyses of manipulated cells.

Group members during 2015

Linda Holmfeldt, researcher, group leader
Karin Gustafsson, researcher
Henrik Steffen, research engineer
Svea Stratmann, PhD student
Ren Sun, post doc

Funding during 2015

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Publications 2013-2015

(The group came to IGP in 2014 and most papers have therefore not been published with IGP as affiliation)

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The Control of Survival and Apoptosis in Human Multiple Myeloma

Helena Jernberg Wiklund

Our research focuses on the hematological malignancy multiple myeloma (MM). MM is a genetically heterogeneous plasma cell malignancy characterized by the accumulation of well-differentiated tumor cells of B cell origin within the bone marrow. To study the molecular mechanisms and therapeutic use of target proteins in survival pathways of MM, a prerequisite has been to select and implement relevant models *in vitro* and *in vivo*. Immunocompetent syngeneic murine models of MM *in vivo*, and *in vitro* models based on a large and well-characterized authenticated panel of cell lines representing all common genetic subtypes of MM, primary patient cells and normal age-matched counterpart cells now constitute our highly clinically relevant model of human MM. New treatment approaches will require research initiatives undertaking novel paths connecting signals of survival within the tumor microenvironment and genetic events, to new mechanisms involving epigenetically regulated gene profiles. Our studies have demonstrated a critical link between IGF-1, a survival factor supporting MM tumor expansion within the bone marrow microenvironment, and epigenetic regulation of transcription *in vitro* and *in vivo*. These findings now provide a clinical rational for epigenetic regulators as candidates for novel therapy (Menu, Jernberg-Wiklund et al. 2006; Stromberg, Ekman et al. 2006; Menu, Jernberg-Wiklund et al. 2007; Kalushkova, Fryknas et al. 2010; Jernberg Wiklund et al 2012; Agarwal, Alzrigat et al. 2016).

Our current goal is to decipher mechanisms promoting abnormal epigenetic silencing acting as a driver of tumor initiation, stemness and drug resistance, with an emphasis on discovering novel targets amenable to future therapeutic intervention in MM.

Identification of genome-wide MM specific epigenetic gene silencing initiated by the Polycomb repressor complex and therapeutic implications of gene reactivation

Antonia Kalushkova, Mohammad Alzrigat, Alba Atienza, Charlotta Sandberg

In our overall aim to dissect the disease-specific global epigenetic pattern of MM, and to evaluate possible links between exogenous survival factors/genetic alterations and the epigenome of MM, we initially undertook an integrative genomics approach on dissecting the differences in gene expression between non-malignant and malignant plasma cells. This novel approach resulted in the seminal finding that a novel common silenced gene profile is present in MM (Kalushkova et al 2010). We have now generated the first global ChIP-seq analysis on the distribution of histone marks including the Polycomb mark H3K27me3 in primary MM cells (Agarwal et al 2016). From this study it is clear that a common and unique epigenetic signature established by the Polycomb exists in multiple myeloma (MM) patient cells. Importantly, this signature is independent from current genetic and molecular sub-classifications of the disease.

Providing a clinical rationale, the signature reflects patients' outcome and tumor burden as defined by ISS staging. This epigenetic signature now stipulates Polycomb as a novel protein complex with oncogenic properties and a potential therapeutic target in MM. We hypothesize, and have provided proof-of-concept, that gene silencing can be reversed and gene expression reactivated by selective inhibitors. Functional studies of targets of epigenetic silencing and consequences of gene reactivation by direct and indirect biological and pharmacological inhibition of Polycomb proteins in MM models *in vitro* and *in vivo* are currently a focus of our investigations.

Novel and combinatorial experimental targeted therapy for MM *in vitro* and *in vivo*

Antonia Kalushkova, Mohammad Alzrigat, Alba Atienza, Charlotta Sandberg

We have previously approached possible targets for therapeutic intervention in MM by studying resistance mechanisms and their combating by evaluating novel rational drug combinations. In line with the assumption that inhibition of any single molecular target will be compensated by concomitant presence of multiple genetic lesions leading to acquired drug tolerance we have explored the role of aberrant NF-κB signaling in hematopoietic tumors including CLL and MM (Mansouri 2015, Fristedt 2015) and provided novel insights on NF-κB regulated transcription (Kalushkova 2016). In this research line we have suggested possible strategies for combinatorial treatment by targeting the NF-κB pathway in sequential treatment with proteasome inhibitors for a subset of MM patients (Fristedt 2015) and emphasized the advantages of combinatorial strategies using mimetics interacting with Bcl-2 survival proteins (BH3, ABT737) (Kharaziha 2012, Karlsson 2013, Bieghs 2014).

Now we are embarking on the evaluation of a panel of epigenetic inhibitors in an intrinsic network of interactions between different epigenetic modifiers that may mitigate the response to inhibitors of Polycomb and maintain persisting gene silencing in MM. We are currently performing a comprehensive genome-wide comparison by aligning the Polycomb enriched regions to methylated CpG sites in MM, and exploring the possibility that demethylating agents at these sites can sensitize MM cells to targeted inhibition of Polycomb proteins.

Alternate metabolite usage underlying resistance to selective epigenetic inhibitors

Antonia Kalushkova, Alba Atienza, Pernilla Martinsson

The field examining the complex interactions between cancer epigenetics and metabolism is fast expanding. We have observed differential sensitivity to epigenetic inhibitors, which impelled us to undertake the approach of using advanced *in vitro* metabolomic mass spectrometry analysis. We base this approach on the fact that previous findings have demonstrated with confidence that metabolite profiles represent highly sensitive markers for phenotypic differences between cells and their responses to drug treatment. To generate a comprehensive "omic" profile of the cellular response to epigenetic inhibition in MM, we are currently generating metabolomic profiles in combination with gene expression analysis in a panel of MM cell lines with differential response. The metabolomic approach will allow us to investigate metabolic changes induced by drug treatment as well as basal differences in metabolic profiles between different entities of MM.

Molecular Networks for Transcriptional Regulation and Epigenetic Control of Differentiation

Fredrik Öberg, Antonia Kalushkova, Mohammad Alzrigat, Aron Skaftason

The project is focused on how epigenetic mechanisms regulate molecular networks with implications for major disease processes, such as the pathogenesis of hematopoietic tumors and chronic inflammation. Mechanisms of epigenetic control are often disturbed in cancer, and aberrant DNA-methylation or histone modifications of specific transcription factor genes, with key functions in the differentiation process, are likely to be important for the pathogenesis of leukemia. Although less well-understood, epigenetic changes are also observed in chronic inflammation and influences disease activity. The data generated by the project will increase the basic knowledge of how epigenetic mechanisms play a role in disease, and discover new target molecules/pathways, amenable to future therapeutic intervention.

The current aims of the project are (1) To investigate the molecular mechanisms for epigenetic reprogramming involved in the control of hematopoietic cell differentiation, (2) To identify genes required to maintain silencing of tumor suppressor genes, and to discover novel compounds with the capacity to relieve epigenetic silencing and reprogram gene expression, (3) To investigate the epigenetic influence on the gene-regulatory network operating in monocytes during chronic inflammation associated with psychiatric illness.
The long-term goal is to achieve a better understanding of the role malignancy-associated epigenetic changes play in perturbing differentiation and activation. In this project we aim at identifying signals or compounds that can re-initiate the blocked differentiation process in hematological malignancies or modulate disease-causing inflammatory activation of monocytes.

Group members during 2015

Helena Jernberg Wiklund, PhD professor, group leader
Antonia Kalushkova, Postdoc
Mohammad Alzrigat, PhD student
Alba Atienza Párraga, PhD student
Charlotte Fristedt Duvefelt, PhD student
Prasoon Agarwal, PhD student
Pernilla Martinsson, research engineer
Charlotta Sandberg, research engineer
Aron Skaftasson, MSc student
Kenneth Nilsson, professor emeritus
Fredrik Öberg, adj associate professor

Dissertations during 2015

Charlotte Fristedt Duvefelt, Tumour Survival Signals and Epigenetic Gene Silencing in Multiple Myeloma: Implications for Biology and Therapy. March 25, 2015.

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Caring Sciences in Oncology Care

Birgitta Johansson

Research in the group focuses on studying how cancer patients feel during and after therapy, and how they have experienced the therapy and care. We also aim to find ways to improve treatment results, reduce toxicity and adverse effects, and improve patients' quality of life.

Each year approximately 55 000 people in Sweden receive a cancer diagnosis. During cancer therapy patients suffer from physical side effects and at the same time the disease often causes psychosocial effects such as anxiety and depression. We are part of several projects that aim to improve care and therapy, and to reduce physical and psychological adverse effects.

Internet based screening and stepped care for adult cancer patients with anxiety or depression symptoms

Catrine Bonnedahl, Anna Hauffman, Marina Forslund, Birgitta Johansson, Madeleine Olsson, Susanne Sjöberg, Peter Nygren,

The research programme U-CARE is an interdisciplinary project in the field of psychosocial care in connection with somatic disease. We are heading the subproject within adult oncology care, which aims to evaluate the effects of internet based stepped care on anxiety, depression and health related quality of life in cancer patients compared to standard care. We also investigate if internet based stepped care is cost-effective and if methods for screening and assessment of anxiety and depression provide clinically meaningful results when administered via Internet.

Effects of an Internet based patient education on patient satisfaction and image quality in ^{18}F-FDG-PET/CT examinations

Camilla Andersson (Surgical Sciences, UU), Birgitta Johansson

^{18}F-FDG-PET/CT is a standard examination used for diagnostics and therapy control in cancer diseases. High image quality from an ^{18}F-FDG-PET/CT examination requires that the patient is well prepared before coming to the examination and that the patient stay still during the examination. Poorly prepared patient can result in that the examination has to be redone.

In this project we analyse the effects of an internet-based patient education about the ^{18}F-FDG-PET/CT examination. We are interested in the effects of the education on patients' satisfaction with the care during the examination and the on the image quality, compared to standard care.

Gastrointestinal symptoms after radiotherapy of prostate cancer

Marina Forslund, Birgitta Johansson, Peter Nygren, Anna Pettersson

This project aims to determine the long-term effects of a dietary intervention on gastrointestinal symptoms after radiotherapy for prostate cancer. Patients were randomized to an intervention group that were advised to reduce insoluble dietary fibre and lactose intake, or to a standard care group advised to continue their usual diet. The main question concern whether patients who receive advise to reduce insoluble dietary fibre and lactose intake report less gastrointestinal symptoms and an improved health-related quality compared to patients who continue their usual diet.

Evaluation of toxicity and care during proton therapy

Birgitta Johansson

The Skandion Clinic in Uppsala is the first clinic for advanced radiation therapy with scanned proton therapy in the Nordic countries. Conventional radiotherapy has several known toxicities but for proton therapy the scientific knowledge regarding patient reported toxicity in short and long term perspectives is scarce. In addition, the patients' experiences of the care during proton therapy have not been investigated.

The main aim of this prospective, longitudinal study is to investigate patient reported toxicity, health related quality of life related to proton therapy in short and long term compared to patients who receive conventional radiotherapy.

The effects of physical exercise during cancer therapy

Birgitta Johansson, Peter Nygren

In the project Phys-Can we are evaluating the efficacy and cost-effectiveness of individually tailored high and low intensity physical training. It is a multi-centre randomized controlled trial including newly diagnosed breast, colorectal and prostate cancer patients during adjuvant therapy in the university hospitals in Uppsala, Lund and Linköping. The main aim is to evaluate the effects of high or low-moderate intensity exercise in combination with behavioural medicine strategies (BM) or without BM on cancer related fatigue.

Group members during 2015

Birgitta Johansson, senior lecturer, group leader
Camilla Andersson, PhD-student (Dept of Surgical Sciences)
Catarine Bonnedahl, research nurse
Marina Forslund, research assistant
Anna Hauffman, PhD student
Peter Nygren, professor
Madeleine Olsson, research nurse
Susanne Sjöberg, research assistant
Associated researchers:
Anna Pettersson
Annika Thalén-Lindström

Funding during 2015

FORTE, 1 000 kSEK,
Swedish Cancer Society, 500 kSEK,
Selander's Foundation, 150 kSEK,
Faculty of Medicine, funding for caring sciences, 400 kSEK
Stiftelsen Onkologiska Klinikens i Uppsala Forskningsfond, 250 kSEK.

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Individualised Cancer Therapy and Development of New Cancer Drugs

Peter Nygren

The main objective of our research is to improve the efficacy of cancer treatment by providing information allowing for optimal drug selection for the individual patient. We also want to identify new compounds with enhanced efficacy against tumour types for which effective therapies are lacking.

Since the start of modern chemotherapy for cancer in the 1940s a number of drugs have become available. However, the doctor's choice of medical treatment generally does not take into account the considerable variation that is known to exist between individual patients in terms of efficacy, tolerance and pharmacokinetics. This means that many patients receive a suboptimal treatment that often only results in side effects.

In recent years new drugs have been developed but the experience so far is that only a small fraction of treated patients might experience good effect of these drugs. At the group level, the benefit is mostly modest.

Our research has two main objectives. One is to is to provide predictive information that allows for optimal drug selection for an individual patient, and, as important, to exclude drugs that will not be active but only produce toxicity.

The second objective, and immediately related to the first, is to identify new lead compounds with potentially improved efficacy against tumour types. Such compounds could be developed into drugs with effect on more patients and for cancer forms that today cannot be treated efficiently.

Identification of small molecules with cytotoxic effects against colorectal cancer tumour cells with specific and clinically relevant mutations

Peter Nygren, Henning Karlsson, Sadia Hassan, Sharmine Mansoori

This is done by screening of drug libraries in colorectal cancer cell line models harbouring defined mutations. This is a collaborative project with Tobias Sjöblom, Dept of Immunology, Genetics and Pathology.

Identification of new drugs that could act synergistically with radiotherapy

Peter Nygren, Henning Karlsson

In this project we investigate interactions between the cytotoxic effects of small molecules and radiation. Candidate drugs have been identified and are now further analysed in 2D and 3D tumour models, to be followed by studies in vivo. The project is performed in collaboration with Rolf Larsson and Mårten Fryknäs, Dept of Medical Sciences.

Testing mebendazole as an anticancer drug in advanced refractory gastrointestinal cancer

Peter Nygren, Malin Berglund

We are planning a phase 1/phase 2a clinical trial of the anti-helmintic drug mebendazole as an anticancer drug in advanced refractory gastrointestinal cancer. The project is based on a pilot study that showed a significant activity of mebendazole in this setting. In parallel, we will investigate the mode of action of mebendazole as an anticancer drug. The project is performed in collaboration with Rolf Larsson and Mårten Fryknäs, Dept of Medical Sciences.

Characterisation of cytotoxic effects of new potential drugs

Peter Nygren, Henning Karlsson, Sadia Hassan, Sharmine Mansoori

Since several years we have been working with an in-house developed short-term in vitro assay for patient tumour cells, the fluorometric microculture cytotoxicity assay (FMCA), which has been shown to report clinically relevant drug activity data in major cancer types. In this project we use the FMCA of the tumour cell to characterise cytotoxic effects of drugs identified in drug repurposing screens in patient tumour samples representing a spectrum of sensitivity to standard drugs. The aim is to identify the tumour diagnoses suitable for future clinical development of these drugs into anticancer drugs.

Group members during 2015

Peter Nygren, professor, group leader
Malin Berglund, technician
Sadia Hassan, researcher
Henning Karlsson, PhD student
Kathrine Bjersand, PhD student
Tanweera Shaheena Khan, physician
Sharmine Mansoori, research assistant
Anne von Heideman, physician

Funding during 2015

Swedish Cancer Society, 600 kSEK
Swedish Research Council, 300 kSEK
Stiftelsen Onkologiska Klinikens i Uppsala Forskningsfond, 200 kSEK

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Molecular Hematology - Chronic Lymphocytic Leukemia

Richard Rosenquist Brandell

The main goals with our translational research program on chronic lymphocytic leukemia (CLL) are to increase our understanding of mechanisms behind disease development, to improve and optimize the diagnostic and prognostic information as well as to reveal new strategies for therapy.

CLL, the most common adult leukemia in Western countries, is a biologically and clinically heterogeneous malignancy with varying disease course. Many patients survive for years or decades even without treatment, whereas others succumb rapidly to the disease despite therapy. Men are more commonly affected than women with a median age at diagnosis of 72 years. At present two staging systems are used in clinical practice (Rai and Binet), however both have a limited ability to predict the clinical course at an early stage. The disease has remained incurable although new treatment strategies, including antibody-based therapy and small molecular inhibitors, appear promising.

In recent years, molecular genetic studies have revealed new prognostic markers, which have significantly improved the subdivision of the disease. Two of the most important molecular predictors are the mutation status of the immunoglobulin heavy variable (*IGHV*) genes and certain recurrent genomic aberrations, which divides CLL into prognostic subgroups.

Impact of stereotyped B-cell receptors in CLL

Lesley-Ann Sutton, Panagiotis Baliakas, Anastasia Hadzidimitriou, Emma Young, Diego Cortese, Sujata Bhoi, Nikos Papakonstantinou, Stavroula Ntoufa, Larry Mansouri, Kostas Stamatopoulos, Richard Rosenquist

An interesting theory that has emerged is the potential role of antigens in the development of CLL. Many reports, including ours, indicate a very biased *IGHV* gene repertoire in CLL, and virtually identical B-cell receptors (BcRs) have been identified in multiple subsets of CLL. In a large collaborative work, we analyzed the complementarity determining region 3 (CDR3) sequences, the main determinant of antigen specificity, in more than 7400 CLL patients, where up to 30% of CLL patients could be assigned to stereotyped subsets. In this study, we proposed a novel molecular classification of CLL based on BcR stereotypy, since patients expressing certain stereotyped BcR appear to have high intra-subset homogeneity both regarding clinical outcome as well as biological features. As an example of the latter point, we demonstrated that subset #2 (IGHV3-21/IGVL3-21) patients exhibit a remarkable 44% frequency of mutations in the *SF3B1* gene, encoding a core component of the spliceosome, whereas other aggressive subsets had frequencies in the range of 0-10%. This finding alludes to subset-biased acquisition of genomic aberrations, perhaps consistent with particular antigen/antibody interactions.

To further investigate the clinical relevance of this new molecular classification based on stereotypy, we performed a multi-center study comprising 8593 CLL patients where individual stereotyped subsets showed profound differences in e.g. demographics, clinical presentation and presence of cytogenetic aberrations. Importantly, members of the same subset followed a similar clinical course, e.g. subsets #1 (IGHV1/5/7/IGKV1-39) and #2 had very short time to treatment and poor overall survival, similar to patients with *TP53* dysfunction, while subset #4 patients (IGHV4-34/IGKV2-30) followed an indolent disease course and were rarely in need of treatment.

We previously demonstrated that CLL subset #4 patients possess distinctive patterns of intraclonal diversification within their IG genes; highly indicative of an ongoing response to

antigen(s). To shed light on the clonal ancestry of subset #4 as a whole, we proceeded to reconstruct their evolutionary history by determining the structure of a community of related clones profiled at different time-points. This approach facilitated for the first time the identification of a common ancestral sequence from which all subset #4 cases are derived. More recently, we have investigated the role of antigens in shaping the T-cell repertoire in CLL, highly relevant in light of the interactions of the CLL B cells with T cells. Indeed, our study of the T-cell receptor beta chain gene repertoire in CLL indicated restriction thereby alluding to antigenic selection.

Refining prognosis and risk stratification in CLL

Larry Mansouri, Lesley-Ann Sutton, Anastasia Hadzidimitriou, Emma Young, Diego Cortese,Viktor Ljungström, Mattias Mattsson, Panagiotis Baliakas, Richard Rosenquist

In a multi-institutional collaborative effort, coordinated under the auspices of the European Research Initiative on CLL (ERIC), we investigated the presence of mutations within *SF3B1, NOTCH1, TP53, BIRC3* and *MYD88*, in the largest cohort ever studied (based on 3490 cases from ten European institutions). We provide strong evidence that different recurrent mutations are associated with distinct clinico-biological profiles and outcomes. The prime example is the finding of *SF3B1* mutations as an adverse indicator among early stage CLL cases, independently of other factors including *TP53* aberrations. We believe that this type of study will be very relevant for the design of future novel prognostic schemes integrating cytogenetic and molecular findings in CLL.

To test the applicability of targeted next-generation sequencing for prognostication, we utilized HaloPlex technology and designed a gene panel including nine prognostic genes: *ATM*, *BIRC3*, *MYD88*, *NOTCH1*, *SF3B1*, *TP53*, *KLHL6*, *POT1* and *XPO1*, and investigated 188 poor-prognostic CLL patients. Sanger validation confirmed 93% (144/155) of mutations; notably, all 11 discordant variants had a variant allele frequency between 11-27%, hence at the detection limit of Sanger sequencing. Technical precision was assessed by repeating the procedure for 63 patients; concordance was found for 94% mutations. We foresee that this new approach will soon be adopted in diagnostics, eventually as a stand-alone test without the need for confirmation by Sanger sequencing.

Novel recurrent gene mutations in clinically aggressive CLL

Lesley-Ann Sutton, Viktor Ljungström, Emma Young, Diego Cortese, Sujata Bhoi, Larry Mansouri, Richard Rosenquist

NF-κB is constitutively activated in CLL, however the implicated molecular mechanisms remain largely unknown. We performed targeted sequencing of 18 core-complex NF-κB genes in 315 cases. The most frequently mutated gene was *NFKBIE* (7% of cases) that encodes IκBε, a negative regulator of NF-κB in B cells. Thirteen of these cases carried a 4bp frame-shift deletion resulting in a truncated protein. The *NFKBIE*-deletion predominated in poor-prognostic patients and was associated with inferior outcome. This truncating mutation resulted in significantly reduced IκBε–p65 interaction and a corresponding increase of p65 phosphorylation compared to wildtype patients. This is the first example of a genetic basis for constitutive NF-κB activation in CLL.

The mechanisms leading to relapse after fludarabine, cyclophosphamide, rituximab (FCR) therapy are incompletely understood. We whole-exome sequenced sequential samples from 41 CLL patients who relapsed after FCR. In addition to recurrently mutated genes i.e. *TP53, NOTCH1, ATM, SF3B1, NFKBIE*, mutations within *RPS15*, a gene encoding a ribosomal component, were identified in 20 % of patients. Analysis of extended cohorts supported a role

for *RPS15* mutations in aggressive CLL. By transiently expressing mutant *RPS15* we found defective regulation of endogenous p53. Hence, we highlighted a novel mechanism underlying clinical aggressiveness in CLL involving a mutated ribosomal protein, potentially representing an early genetic lesion in CLL pathobiology.

Group members during 2015

Richard Rosenquist Brandell, professor, group leader
Panagiotis Baliakas, PhD student
Sujata Bhoi, PhD student
Diego Cortese, PhD student
Karin Larsson, physician
Viktor Ljungström, PhD student (also in Tobias Sjöblom's group)
Larry Mansouri, researcher
Mattias Mattsson, PhD student
Stavroula Ntoufa, post doc
Nikos Papakonstantinou, post doc
Kostas Stamatopoulos, guest professor
Christer Sundström, professor emeritus
Lesley-Ann Sutton, post doc
Aliki Xochelli, post doc
Emma Young, PhD student

Funding during 2015

Swedish Cancer Society, 1 250 kSEK
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Identifying and understanding mutations causing colorectal cancers

Tobias Sjöblom

We aim at finding and understanding somatic mutations that cause common human cancers, particularly colorectal cancers (CRC) (Sjöblom *et al*, *Science* 2006). By studying these mutated genes using forward and reverse genetic approaches in human cancer cells, we want to understand their contribution to tumor development. The findings may aid in development of methods for early tumor detection, improved diagnosis, and targeted cancer chemotherapy.

Integrated data and sample collection in clinical cancer care

Tony Hansson, Lucy Mathot, Maria Karoutsou, Emelie Bladin

Identification of mutated genes that cause cancer or resistance to cancer therapies requires systematic sample collection from cancer patients. With support from the Swedish Government, we coordinate an open access longitudinal collection of patient data, tissues, and imaging before, during, and after cancer therapy at Uppsala Academic Hospital and Umeå University Hospital (www.u-can.uu.se). At the end of 2015, more than 8,000 patients with cancers of the colorectum, brain, prostate, ovaries, neuroendocrine tissues, breast, lung, lymphoma or haematological malignancies had been included in U-CAN (Tobias Sjöblom, Program Director; Tony Hansson, Administrative Director; and U-CAN clinical partners). U-CAN received Excellence reviews in the three different evaluation criteria in the external evaluation of Strategic Research Areas (SFO/SRAs) of 2015 and will continue to support patient inclusion but gradually re-focus to increase the information density and quality for each patient and support research based on the collected materials. Near 200 tumors from patients in U-CAN have undergone whole genome sequencing and several biomarker studies have been initiated by different research groups. The first publication encompassing patients from U-CAN was recently published (Ljungström et al, *Blood* 2016).

Major constraints on cancer genomics include obtaining DNA from the large patient cohorts required to gain knowledge about infrequently mutated genes, and the need for improved extraction technologies in diagnostic molecular pathology. We have therefore developed, patented and automated a technology for scalable serial extraction of DNA and RNA from tissue samples (Mathot *et al*, 2011; Mathot *et al*, 2013). The spin-out company ExScale Biospecimen Solutions AB, founded in 2012, has now completed development and CE/IVD labelling of a reagent system for automated serial extraction of DNA and RNA from FFPE samples in clinical diagnostics and launched products on the market in 2015.

Mutational studies of candidate cancer genes

Tom Adlerteg, Ivaylo Stoimenov, Lucy Mathot, Viktor Ljungström, Veronica Rendo

By comparing DNA sequences in cancer genomes to sequences in the constitutional genome of the same patient we can derive somatic mutations that have been acquired during tumor evolution. Such somatic mutations are the basis for modern cancer diagnostics and therapeutics development. We have determined the nucleotide sequences of 37 candidate breast cancer genes previously discovered by us, and identified novel mutations in 12 genes of which *DIP2C* is subject to further functional studies (Jiao *et al*, 2012; Larsson *et al*, manuscript in submission). To visualize mutations in tumors, we adapted *in situ* padlock probes for use in FFPE tissues (Grundberg *et al*, 2013) and applied the technology for the first *in situ* mutational analyses of *TMPRSS-ERG* rearrangements in human prostate cancer tissues (Kiflemariam *et al*, 2014). Further, we have developed software tools for rapid and accurate mutational analysis of deep sequencing data from solid tumors with significant content of

normal cells. These tools have superior indel calling capabilities, a major challenge in mutational analysis, as compared to state of the art (Stoimenov, Adlerteg *et al*, manuscript). For this application, novel statistical mathematics has been developed which has also been patented (Swaminathan et al, submitted).

Using these tools, we have completed deep mutational analyses of 676 genes in cancer pathways in 107 colorectal cancers (Mathot, Ljungström *et al*, manuscript). While the expected frequencies and types of mutations were observed in known CRC genes such as *APC*, *KRAS*, and *TP53*, we noted an enrichment of mutations in the Ephrin receptor tyrosine kinase gene family in tumors giving rise to metastasis. Ephrin receptors have previously been associated with metastatic disease development due to their role in tumor growth, invasiveness, angiogenesis and metastasis *in vivo*. However, no mutational evidence has yet been presented to explain the downregulation of Eph proteins associated with metastasis of CRCs. Functional data indicate that these mutations confer a phenotype on colorectal cancer cells (Mathot *et al*, manuscript).

The use of targeted deep sequencing in this study meant that we could uncover low frequency variants that would otherwise be overlooked. These findings are potentially of great clinical importance to identify patients that require close monitoring to detect recurrence and to stratify CRC patients that would benefit most from adjuvant treatments. Current efforts include whole genome sequencing of CRC cases in U-CAN where longitudinal blood samples are available.

Functional studies of novel candidate cancer genes

Tatjana Pandzic, Snehangshu Kundu, Chatarina Larsson, Ivaylo Stoimenov, Sara Kiflemariam, Veronica Rendo

Gene mutation prevalence is a strong indicator of selection during tumor development, but does not suffice to prove cancer gene status - functional and phenotypic studies comparing mutant and wild-type alleles in relevant model systems are required for ultimate proof. One approach to perform such analyses is through genome editing in human cancer cells. We have developed scalable experimental and computational tools for designing rAAV gene targeting constructs to all genes in the human genome (Stoimenov, Akhtar Ali *et al*, *NAR*, 2015). This technology was used to knock out the putative breast cancer gene *DIP2C* and obtained evidence for a phenotype linked to gene inactivation, and identified more than 700 genes with altered expression, many of which affect cell proliferation (Larsson *et al*, manuscript in submission). We have targeted the transcriptional modulator *ZBED6* in colorectal cancer cells and demonstrated effects on cell growth rate and regulation of genes in CRC pathways (Akhtar Ali *et al*, *Proc Natl Acad Sci* 2015). We have also generated knock-ins of colorectal cancer genes (*PRDM2*, *MLL3*, and *KRAS*) that are currently being characterized by us and used by collaborators in drug discovery efforts (Pandzic *et al*, in review; Larsson *et al*, Akhtar Ali *et al*, manuscripts).

While many low frequency cancer genes (mutated in 1-5 % of patient cases) have been discovered by large scale sequencing efforts, their involvement in cancer pathways and phenotypes is often less clear. To better understand which genes belong to the Ras pathway in human CRC, we have adapted technology for forward genetics by transposon mutagenesis in human cells to map the RAS pathway in human colorectal cancers by a phenotypic screen. This resulted in assignment of 163 recurringly targeted genes to the Ras pathway. After comparing with mutational analyses of human colorectal cancer genomes and performing mutual exclusivity analysis with *KRAS*/*BRAF*, 15 genes were selected for further validation. Of these, 3 genes showed changes in GLUT1 expression after knock-down and differential growth in low glucose, phenotypes associated with Ras pathway activation in CRC. Two of

the three genes controlled the level of pERK in CRC cells, providing independent evidence of them being components of the Ras pathway (Kundu *et al*, submitted).

The tissue expression patterns of cancer genes may yield insights into the anatomy of cancer pathways and the expression profiles of cancer drug targets. *In situ* hybridization (ISH) offers a scalable and specific approach to mapping gene expression in tissues, and we have therefore established and automated large scale ISH on FFPE tissue arrays. We have evaluated the expression patterns of the tyrosine kinome and the tyrosine phosphatome in ~40 normal tissues and 6 common tumor types, totalling 37 000 tissue specimens, leading to the discovery of novel tumor specific stromal and vessel biomarkers in human cancers (Kiflemariam *et al*, *Am J Pathol* 2015). Among these findings, tumor vessel specific up-regulation of INSR was uncovered.

Exploiting loss of heterozygosity for a novel anti-cancer therapy

Veronica Rendo, Ivaylo Stoimenov

The success of any anti-cancer therapy is based on finding conditions resulting in selective killing of cancer cells, while the normal tissues of the patient are spared. As an alternative to the existing strategies we propose a conceptually different therapy, which exploits the genetic variation (SNPs) naturally occurring in the human population and the cancer specific phenomenon loss of heterozygosity (LOH). For example, if the patient is constitutionally heterozygous for a high efficiency allele and a low efficiency allele, and the tumour loses the high efficiency allele through LOH, it is conceivable that the tumor is sensitized to certain drugs relative to the normal tissues.

Using 1000 Genomes data, we identified human enzymes having variant amino acids in their active sites as result of SNPs and ranked the 20 putative targets according to the prevalence of SNPs and LOH in common human cancers. For the top candidate, a known drug metabolic enzyme, we estimate that >3 % of patients with CRC could benefit from a tailored drug therapy, which translates to >35,000 cases worldwide per year. We therefore constructed and validated CRC cell model systems for cell based drug screens for the most promising candidate, NAT2, in two independent genetic backgrounds. Subsequent drug discovery efforts uncovered a compound with 5-fold increased cytotoxic potency in cells lacking NAT2, which was confirmed to be a NAT2 specific substrate *in vitro*. Current efforts include in vivo evaluation of cytotoxic activity, high throughput screening for additional hit compounds and assessment of targetability of other common NAT2 variant alleles (Ivaylo Stoimenov, Veronica Rendo).

A similar project where inactivating polymorphisms (STOPs and indels) have been systematically investigated led to the identification of a promising candidate target enzyme for which cell models have been developed and compound screening will start in 2016 (Veronica Rendo, Ivaylo Stoimenov).

Group members during 2015

Tobias Sjöblom, senior lecturer, group leader
Tom Adlerteg, research engineer
Emelie Bladin, research engineer
Per Carlstedt, degree project student
Erik Hallqvist Osterman, teaching assistant
Tony Hansson, project coordinator
Karin Hartman, research assistant
Snehangshu Kundu, researcher
Chatarina Larsson, researcher

Viktor Ljungström, PhD student (also in R Rosenquist's group)
Lucy Mathot, PhD student
Tatiana Pandzik, researcher
Veronica Rendo, PhD student
Ivaylo Stoimenov, post doc

Funding during 2015

Swedish Cancer Society, 500 kSEK
Swedish Cancer Society, 650 kSEK (postdoc fellowship for C. Larsson)
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Johanssons foundation, 100 kSEK (to C. Larsson)
Swedish Research Council, 300 kSEK (PI: U. Landegren)
ExScale Biospecimen Solutions AB, 500 kSEK (commissioned research)

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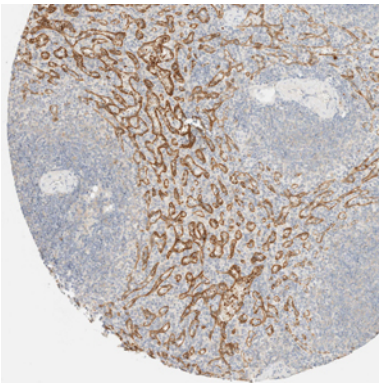
Human Protein Atlas



The aim of the Swedish Human Protein Atlas is to determine gene expression patterns on both mRNA and protein levels in human normal and diseased tissues and cells; to systematically generate a map showing the distribution and relative abundance of all human proteins and to present all data on a freely available web portal (www.proteinatlas.org). In addition, the Human Protein Atlas provides a starting point for translational biomedical research including the discovery and validation of potential clinical cancer biomarkers.

The Human Protein Atlas

Fredrik Pontén, Mathias Uhlén

The Swedish Human Protein Atlas project has been set up to allow for a systematic exploration of the human proteome using an antibody-based proteomics strategy. This mapping effort can be viewed as an ambition to generate an additional ”layer” of information on top of the human genome sequence data. By determining the localization and relative abundance of proteins in specific tissues, cells or subcellular compartments our general knowledge will increase. There is also a demand for new biomarkers, particularly in the field of cancer diagnostics where markers are needed to determine cellular differentiation, grade of malignancy and stratification of tumors with respect to prognosis and response to therapy.

Analysis of protein expression patterns is performed using immunohistochemistry on tissue and cell microarrays. These contain more than seven hundred spots of normal and cancer tissues as well as *in vitro* cultured cells. Immunohistochemically stained tissue microarray sections are scanned to obtain high-resolution images. Each image is manually annotated to determine expression and localization profiles. Cells are annotated using an image analysis-based system.

In addition to generating antibody-based protein profiling data, the Human Protein Atlas has also performed transcriptomic (RNA-seq) analyses for the majority of tissues and cell-lines used in the project. This transcriptomic data is integrated to the atlas to provide an additional layer of information of gene/protein expression in our tissues, and furthermore serves as a tool to validate the proteomic data generated by antibodies.

All protein and RNA profiling data, including the underlying high-resolution images is presented in an anatomically comprehensive, publicly available protein atlas (www.proteinatlas.org). New data and more features are released in annual updates of the database. The current version 15 of the Human Protein Atlas includes protein profiles from close to 25,000 antibodies generated towards 17,000 unique proteins (corresponding to over 80 % of the human protein encoding genes). All antibodies are used for protein profiling in normal human tissues from 144 individuals, where a defined set of normal cell types are annotated for each tissue, and in 216 different tumors representing the 20 most common forms of human cancer. In addition to the high throughput protein profiling core project, several projects with more specific objectives are run based on the resources gene-rated within the Human Protein Atlas.

Below is a short description of such selected projects, in which the work of the technical staff headed by Cecilia Lindskog-Bergström should also be acknowledged.

Cancer biomarkers

Per-Henrik Edqvist, Kristina Magnusson, Anna Asplund, Caroline Kampf, Cecilia Lindskog, Julia Bergman-Larsson, Fredrik Pontén

In collaboration with Karin Jirström (MAS), Patrik Micke, Johan Botling, Irina Alafuzoff, Michael Bergqvist, Anja Smiths, Anca Dragomir, Bengt Glimelius (AS), Dan Hellberg (Falu lasarett), Lars Holmberg (ROC), Monica Nistér, Georg Klein (KI), Jutta Huvila, Olli Carpén (Turku University), Irma Fredriksson (KI/KS), Anna Dimberg (UU), Gabriella Gremel (Manchester University, UK), Gillian O'Hurley (Oncomark, Dublin), Halfdan Sörbye (Bergen University Hospital, Norway), Camilla Qvortrup, Per Pfeifer (Syddansk universitet, Denmark), Mathias Uhlén (SciLifeLab).

In several projects the aim is to further analyze the role of proteins identified as potential cancer biomarkers in the screening effort performed within the Human Protein Atlas project. Tumor material from well-defined patient cohorts, with tumors representing all major forms of human cancer, are being collected and assembled into tissue microarrays. In addition to tumor material, clinical data is also collected to create databases allowing for testing and validation of protein expression patterns of importance for diagnostics, prognostics and functional tumor biology studies. There is special emphasis on i) lung cancer for identification of prognostic and treatment predictive markers, ii) colorectal cancer, U-CAN cohort, for the identification of markers that can stratify patients into groups of high or low risk for recurrent disease, iii) breast cancer in young women based on a large national cohort and extensive clinical database of >1000 patients where the focus is to understand why this patient group has such poor prognosis, and iv) gynecologic cancers for evaluation of novel prognostic biomarkers. Other collaborative biomarker projects include melanoma, high and low grade gliomas, cervical cancer and prostate cancer.

Tissue specific proteomes defined by RNA-seq and antibody-based protein profiling

Cecilia Lindskog-Bergström, Sandra Andersson, Dijana Djureinovic, Linda Oskarsson, Evelina Sjöstedt, Angelika Danielsson, Per-Henrik Edqvist, Anna Asplund, Agata Zieba, Caroline Kampf, Julia Bergman-Larsson, Fredrik Pontén

In collaboration with Uppsala Akademiska Hospital, Dept. of Clinical Pathology, Linn Fagerberg, Björn Hallström, Jan Mulder (SciLifeLab), Åsa Sivertsson (KTH), Gabriella Gremel (Manchester University, UK), Karolina Edlund (Ifado, Tyskland), Mathias Uhlén (SciLifeLab)

The large-scale RNA-seq effort of multiple human normal tissues undertaken by the Protein Atlas project has facilitated the systematic comparison among tissues with the aim of defining the "tissue-specific proteome" for each tissue. The project is focused on identifying the highest abundant tissue-enriched or group-enriched transcripts (for highly similar tissues) and comparing these across all other tissues or tissue-groups. The antibody-based IHC protein profiling data is included in these analyses to provide a spatial resolution of where the gene is expressed on the protein level with respect to different cell types/ sub-compartment/layers, etc. On a global scale, over 20 such tissue- or tissue-group specific proteomes have been defined to date.

Novel diagnostic tools for determining the origin of cancer metastases

Julia Bergman, Dijana Djureinovic, Per-Henrik Edqvist, Fredrik Pontén

In collaboration with Karin Jirström (MAS), Patrick Micke (UAS), Gabriella Gremel (Manchester University, UK), Mathias Uhlén (SciLifeLab)

The use of antibodies that target proteins that are tissue- or cell type specific are crucial diagnostic tools in clinical pathology where they are used in immunohistochemistry-applications for the characterization of cancer. Such specific diagnostic antibodies can be used to determine from which original tissue the cancer has developed and to sub-classify the tumor type. The vast amount of data in the Human Protein Atlas is screened for cell and tissue specific proteins. Identified candidates are further validated and characterized for sensitivity and selectivity of specific target binding. Selected antibodies are used to analyze the expression pattern in a large TMA (over 900 cases) containing mainly metastases and primary tumor tissue from tumor types where additional diagnostic markers are needed. The aim is to find and define panels of diagnostic markers to be used in clinical pathology.

Protein profiling using highly characterized antibodies towards cancer proteins

Per-Henrik Edqvist, Evelina Sjöstedt, Cecilia Lindskog Bergström, Fredrik Pontén

In collaboration with Gordon Whitely, Stephen Hewitt (NCI-CPTC program), Mathias Uhlén (SciLifeLab)

In an effort to generate highly characterized monoclonal antibodies towards proteins suggested to be involved in cancer growth and spread, the NCI initiated the CPTC program to drive the development of a central community core that would help accelerate biomarker discovery and validation, cancer diagnostics development, and therapeutics monitoring. As part of this effort CPTC antibodies are tested and used for protein profiling using Human Protein Atlas strategies including immunohistochemistry and immunofluorescence.

Group members during 2015

Fredrik Pontén, professor, group leader
Groom Alemayehu, research engineer
Sandra Andersson, PhD
Maria Aronsson, research engineer
Anna Asplund, PhD, researcher
Julia Bergman-Larsson, PhD student
Cecilia Lindskog Bergström, PhD, deputy site director
Dijana Djureinovic, research engineer
Anca Dragomir, physician
Per-Henrik Edqvist, assoc. prof., project coordinator
Feria Hikmet Noraddin, research engineer
Caroline Kampf, assoc. prof., site director
Borbala Katona, research engineer
Dennis Kesti, research assistant
Lillemor Källström, lab technician
Erik Larsson, adj. professor
Erik Lindahl, research assistant
Kristina Magnusson, PhD
IngMarie Olsson, research engineer
Marcus Runeson, research engineer
Evelina Sjöstedt, research engineer
Jimmy Vuu, research engineer

Dissertations during 2015

Sandra Andersson, Validation of antibodies for tissue based immunoassays. June 13, 2015.

Kristina Magnusson, Protein Expression Profiling of Cancer Biomarkers. December 18, 2015.

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Knut and Alice Wallenberg foundation, 8 300 kSEK
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Medical Genetics and Genomics

The research groups in this program are addressing basic mechanisms in genetics, epigenetics and genomics as well as more applied questions in clinical genetics, genetic epidemiology, cancer genetics and forensic genetics.

We use methods that can identify differences in single genes as well as in our genome as a whole. The aim is to understand the function of our genome and to identify causes of metabolic diseases, cancer, neurodevelopmental disorders and congenital malformations. Studies on the genetic variability of the human genome will also increase our knowledge of our evolutionary origin. New methods for forensic DNA testing are developed to allow analysis of challenging samples from crime scenes.

Improved Forensic DNA Analysis

Marie Allen

The general objective of our research is to develop highly sensitive and discriminating assays for forensic DNA analysis of challenging samples. Evidence samples at a crime scene have often been subjected to harsh environments and have therefore commonly degraded DNA that may also be present in very small amounts. Our research involves development of quantification assays and typing systems for analysis of mitochondrial DNA (mtDNA) as well as autosomal markers. In addition, a Y-chromosome analysis can allow resolution of mixed DNA samples (common for instance in sex offence cases). The use of mtDNA markers will allow a highly sensitive analysis due to a high copy number of mtDNA molecules per cell, while the autosomal markers in very short fragments will give a high discrimination power.

Several new assays based on pyrosequencing, microarrays, real-time quantification or Sanger-sequencing have been developed and used successfully in analysis of challenging evidence material in forensic cases. In a new study, a combination of traditional methods and next generation sequencing (NGS) technologies will be evaluated for DNA analysis of degraded, limited and damaged samples. A target selection and enrichment is performed using Agilent's HaloPlex system for customized panels of a large number of targets that is based on a capture technology with high sensitivity. The MiSeq sequencer will be used for the final sequence analysis. This strategy will allow high throughput analysis of multiple markers in the genome and will allow improved relationship analysis, prediction of visible characteristics and individual identification. In general, new identification assays allow smaller amounts and also degraded DNA to be analysed. As an ultimate test for success with challenging samples, the novel techniques may be used in genetic investigations of historical samples.

Saint Birgitta (Saint Bridget of Sweden) lived between 1303 and 1373 and was appointed one of Europe's six patron saints by the Pope in 1999. According to legend, the skulls of St. Birgitta and her daughter Katarina are maintained in a relic shrine in Vadstena abbey in mid Sweden. The authenticity of the two skulls was assessed first by analysis of mitochondrial DNA (mtDNA) that excluded a maternal relationship. Moreover, a radiocarbon dating suggest an age difference of at least 200 years and neither of the dating results coincides with the period St. Birgitta or her daughter Katarina lived. Similarly, we have performed DNA

analyses using novel sensitive assays to identify the remains of Nicolaus Copernicus and Carin Göring.

Group members during 2015

Marie Allen, professor, group leader
Magdalena Bus, post doc
Martina Nilsson, project manager

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Publications 2013-2015

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Characterisation of Syndromes Associated with Developmental Delay

Marie-Louise Bondeson

Developmental delay, with or without malformations, occurs among two to three percent of the population. For approximately half of the patients the reason for the developmental delay is still unknown, despite extensive studies. Knowledge about the genetic causes of the syndromes is important for diagnosis, prognosis, treatment and risk for recurrence. It will also increase our understanding of the molecular processes behind the disorders.

Our research projects concern Down syndrome, characterisation of novel syndromes, RASopathies, including e.g. Noonan syndrome and intra uterine fetal death (IUFD) and We also have a project that focuses on genetically caused hearing loss. The research is performed in collaboration with physicians and researchers at the Uppsala University Hospital.

Down syndrome: epidemiological, clinical and molecular characterisation:

Ulrika Wester-Oxelgren, Göran Annerén,

In collaboration with Jan Gustafsson, Åsa Myrelid (UU)

Down syndrome (DS) is the most common cause for developmental delay. Patients with DS have, besides developmental delay, an increased risk of being afflicted by several other diseases. DS is a model disease for studies on the relationship between chromosome imbalance and disease. Our research includes epidemiological, clinical, as well as molecular genetic studies of the disorder.

The specific aims of this project are:

- To perform a genotype-phenotype correlation of gene-dosage effects on chromosome 21. The studies have so far been focused on aging and dementia in DS in relation to gene-dosage effects of SOD and APP and autoimmune reactions in relation to the gene-dosage effect of the *AIRE* gene and the mental retardation in relation to the *DYRK1A* gene Studies are also in progress to study the effect of medications on ADHD
- To study the prevalence of ADHD and Autism spectrum disorders in a population of DS and to study the effects of specific treatments of the disorders.
- To study the *DYRK1A* gene on neuronal IPS cells from patients with DS and to study the effect upon those cells from treatment with Harmine.

Characterisation of novel syndromes using microarray-analysis and next generation sequencing

Marie-Louise Bondeson, Christian Wentzel, Cecilia Soussi Zander, Göran Annerén, Sanna Gudmundsson , Ann-Charlotte Thuresson

Intellectual and developmental disorders (IDD) are one of the main reasons for referral in paediatric, child-neurological and clinical genetic service. We are using array based technologies to screen the genomes of patients for chromosomal aberrations to identify the underlying mechanism to possibly categorise new syndromes and genes associated with IDD. In selected groups of patients, where no chromosomal aberration has been detected, next generation sequencing (NGS) technologies are used to screen genomes of patients at high resolution to identify new causative genes for IDD.

Clinical and molecular characterization of Noonan spectrum disorders (RASopathies)

Sara Ekvall, Berivan Baskin, Cecilia Soussi Zander, Göran Annerén, Marie-Louise Bondeson

Recent advances in molecular genetic research have led to the definition of a new group of genetic syndromes, the RAS/MAPK pathway disorders or ”RASopathies”. They comprise Noonan syndrome and related disorders (Noonan with multiple lentigines, Cardio-faico-cutaneous and Costello syndromes), as well as Neurofibromatosis type 1. The aim of this study is to enable translational research into disease mechanism and therapies of the RASopathies. The RAS/MAPK pathway, which has been well studied in cancer, is an attractive target for inhibition in the treatment of various malignancies utilizing small molecule therapeutics, which specifically inhibit the pathway. The specific aims of the project are to identify novel causative genes associated with RASopathies using NGS and to investigate the functional role of different mutations in the RAS/MAPK pathway to clarify the underlying molecular mechanisms.

Clinical and molecular characterisation of intrauterine fetal death (IUFD)

Maria Wilbe, Sanna Gudmundsson, Carina Frykholm, Katharina Ericson, Marie-Louise Bondeson

Each week, nine stillborn children are born in Sweden, implicating that around 450 children/year die in the end of the pregnancy. A large spectrum of pathogenic processes can lead to fetal death and the genetic etiology is unknown in the majority of cases. This causes an enormous suffering for affected families. In a recent study only 25-27% of fetuses with congenital malformation syndromes could be genetically diagnosed.

This research project aims to identify and characterize the genetic abnormalities that can cause fetal death by examining the genome in affected families. Families recruited to the study have been comprehensively investigated genetically and as a last option, participation in this research project is offered. This is a translational research project that will apply findings from basic research (discover genetic causes of IUFD and identify novel pathways and genes important for development and embryogenesis) to enhance human health and well-being, by improving diagnostics and implementations into clinics.

Group members during 2015

Marie-Louise Bondeson, professor, clinical molecular geneticist, group leader
Göran Annerén, professor em., senior clinical geneticst
Berivan Baskin, assoc. prof., clinical molecular geneticist
Sara Ekvall, researcher
Carina Frykholm, clinical geneticist
Sanna Gudmundsson, PhD student
Cecilia Soussi Zander, clinical geneticist
Ann-Charlotte Thuresson, assoc. prof., clinical molecular geneticist
Christian Wentzel, clinical geneticist
Maria Wilbe, researcher
Ulrika Wester-Oxelgren, MD, PhD student

Funding during 2015

ALF, 610 kSEK
Borgström foundation, 100 kSEK
Börjeson E&R foundation, 264 kSEK
Erik Philip Sörensson foundation, 240 kSEK
The Nilsson-Ehle-endowments, 130 kSEK
Medical faculty, Uppsala University, 264 kSEK
Sävstaholm foundation, 425 kSEK

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Novel Mechanisms, Models and Therapeutic Targets for Inherited Disorders

Niklas Dahl

The purpose of this project is *i)* to identify novel phenotypes and genetic mechanisms behind disorders, mainly with a Mendelian inheritance *ii)* to explore iPSC derived systems to model human diseases and *iii)* to identify biomarkers and pathophysiological mechanisms that may serve as targets for diagnostics and for rescue screening of small compound libraries.
The long term goal is to generate knowledge and conditions for the development of novel therapeutic strategies in disorders of this study.

Disease associated gene variants: Analysis of functional effects and high trough-put Omics analysis

Joakim Klar, Maria Sobol, Doroteya Raykova, Muhammad Jameel, Zafar Ali, Jens Schuster

More than 100 extended pedigrees segregating various disorders, mainly of Pakistani origin, have been identified and sampled. Clinical information and family structures have been clarified. All families have multiple affected individuals segregating unique or rare autosomal recessive or autosomal dominant traits. The phenotypes are variable and approximately half of the families present with symptoms from the central nervous system (CNS). To date, we have performed whole exome sequencing (WES) in >100 individuals with unclear Mendelian traits. Our yield of confirmed or likely disease causing gene mutations is 74% in all families analysed. We recently identified several novel candidate genes for disease, e.g. *TBCD* and *MAST2* genes (in severe intellectual disability and cerebral palsy, respectively) as well as *Claudin10b* gene (in kidney disease and isolated anhidrosis). The *TBCD* and *MAST2* genes encode proteins that interacts with β- and α- tubulin.

In parallel, we have analysed the methylomes, transcriptomes, proteomes and metabolomes in cells derived from selected patients/disorders and genetic variants. The combined analyses have resulted in novel findings that are now being processed.

Functional parameters associated with candidate gene variants are tested using cell systems and model organisms. As examples we have edited orthologous genes in zebrafish using CRISPR/Cas9 to clarify the role of tubulin-associated proteins MAST2 and TBCD in CNS development and intellectual disability. Furthermore, we have recently clarified the role for *Claudin10b* variants in kidney disease: Preliminary analysis of HEK and MDCK cells expressing mutated Claudin10b protein show loss of tight junction formation with a resulting loss of affinity for Na^+-ions in the paracellular space.

Induced pluripotent stem (iPS) cells for disease modeling: Functional analysis of disease mechanisms in neurological disorders

Loora Laan, Maria Sobol, Jens Schuster, Doroteya Raykova, Ayda Khalfallah, Joakim Klar

Neurodevelopmental disorders affect approximately 2 % of the population. Little is known about the mechanisms leading to early neuronal defects in the central nervous system. Induced pluripotent stem cell (iPSC) technology has the capacity to recapitulate lineage specific development and pathophysiology. Human skin fibroblasts derived from patients with clinically well-defined neurodevelopmental disorders are reprogrammed to iPSC using a non-integrating vector system. The iPSC are differentiated into different neuronal lineages using established protocols. We have so far focused on modeling the neurodevelopment and

pathophysiology in Dravet disease (therapy resistant epilepsy) caused by mutations in the *SCN1A* gene; Mowat-Wilson syndrome (severe intellectual disability and craniofacial dysmorphism) caused by mutations in the *ZEB2* gene and Down syndrome (intellectual disability, metabolic changes, malformations) caused by trisomy 21. The three disorders are selected as they represent different aspects of aberrant CNS development and without efficient treatment options.

Differentiated neuronal cells are analysed for different multi-omics modalities using SciLife platforms (transcriptome- proteome- and metabolome profiles) as well as growth, migration, neurite formation and electrophysiology for the identification of disease-associated alterations. The combined analyses have resulted in novel findings that are now being processed and validated. Long-term bioinformatic support has been approved by the Wallenberg Foundation (WABI). The goal is to identify biological disease markers in early steps of differentiation and to rescue these abnormalities in a screening platform available at Chemical Biology Consortium Sweden (CBCS).

Group members during 2015

Niklas Dahl, professor, group leader
Zafar Ali, guest PhD student
Angêlica Delgado Vega, physician
Ayda Khalfallah, post doc
Joakim Klar, researcher
Loora Laan, PhD student
George Maronitis, degree project student
Doroteya Raykova, PhD student
Jens Schuster, researcher
Maria Sobol, post-doc
Eva-Lena Stattin, physician/researcher

Dissertations during 2015

Doroteya Raykova, Genetics of Two Mendelian Traits and Validation of Induced Pluripotent Stem Cell (iPSC) Technology for Disease Modeling, April 24, 2015.

Funding during 2015

Swedish Research Council, 900 kSEK
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Molecular Oncology

Jan Dumanski

Analysis of post-zygotic or somatic genetic variation (somatic mosaicism) in normal cells is the overall theme of research in the group. We work with translational disease-related projects and with basic questions addressing somatic variation in normal human cells. An emphasis is on structural genetic variation, which has emerged over the past 10 years as a dominating type of human inter-individual variation.

Mosaic loss of chromosome Y (LOY) in blood cells is associated with smoking as well as shorter survival and higher risk of cancer in men

Lars A. Forsberg, Chiara Rasi, Hanna Davies, Edyta Rychlicka, Jan Dumanski

LOY and cancer

It is well known that men have an overall shorter life expectancy compared with women. However, it is less well recognized that incidence and mortality for sex-unspecific cancers are higher in men, a fact that is largely unexplained. Age-related loss of chromosome Y (LOY) is frequent in normal hematopoietic cells and it was first described more than 50 years ago, but the phenotypic consequences of LOY have been elusive. Our latest results suggest that LOY could be a key factor to explain the higher mortality of men.

Survival analyzes performed in the Swedish ULSAM-cohort (Uppsala Longitudinal Study of Adult Men) with >1100 participants indicated that LOY in peripheral blood could be associated with risks of all-cause mortality as well as non-hematological cancer mortality. Among the elderly men in this cohort, followed clinically for up to 20 years, at least 8.2% of the subjects were affected by LOY in a significant fraction of blood cells. The median survival time in men affected with LOY was half, i.e. 5.5 years shorter, compared to the men without mosaic LOY in blood cells. The association of LOY with risk of all-cause mortality was validated in the independent PIVUS-cohort (Prospective Investigation of the Vasculature in Uppsala Seniors) in which 20.5% of men showed LOY. Our discovery of a correlation between LOY and all-cause mortality as well as non-hematological cancer mortality will be published in Nature Genetics.

These results illustrate the impact of post-zygotic mosaicism such as loss of chromosome Y (LOY) on disease risk and could explain why males have a higher mortality compared to females and are more frequently affected by cancer. They also suggests that chromosome Y is important in processes beyond sex determination and sperm production. LOY in blood could become a predictive biomarker of male carcinogenesis.

LOY and smoking

Smoking is a major preventable environmental risk factor related to human health. Smoking killed about 100 million people during the 20th century and is projected to kill one billion people during this century, assuming that the current frequency of smoking is retained. Lung cancer is the prime cause of cancer associated death in relation to smoking. However, it is less well appreciated that smoking also causes tumors outside the respiratory tract, which is predominant in men and cumulatively roughly as common as lung cancer. Moreover, it is known that males have a higher incidence and mortality from most sex-unspecific cancers, disregarding smoking status, and this fact is largely unexplained by known risk factors.

We have published a paper in *Science* showing that smoking is associated with and LOY in blood cells in three independent cohorts encompassing in total 6014 men. Our data also support a transient and dose-dependent mutagenic effect from smoking on LOY-status (Dumanski et al. 2015 Science, PMID: 25477213). Thus, smoking may induce LOY, linking

the most common acquired human mutation with a severe preventable risk factor. Our results could explain the observed sex differences and why smoking seems a greater risk factor for cancer in men than women.

Post-zygotic genetic variation: studies of human aging/longevity and age-associated aberrations

Lars A. Forsberg, Chiara Rasi, Jan Dumanski

In collaboration with Lars Lannfelt, Martin Ingelsson, Erik Ingelsson, Lars Lind et al. (UU).

Monozygotic (MZ) twins represent an extraordinary resource in genetics; two individuals who can also be treated as a single subject genetically matched at conception and present in two copies. Therefore, it is a powerful model for analysis of *de novo* (post-zygotic or somatic) genetic variation. We have shown in 2008 that MZ twins frequently display disparate patterns of genomic copy number variation (CNV). We hypothesized that structural genetic rearrangements in human somatic cells also vary over time and these might represent a new mechanism contributing to the aging process in humans.

Using age-stratified cohorts of 318 monozygotic (MZ) twins and 296 single-born subjects, we found age-related accumulation of copy-number variation in the nuclear genomes in vivo and frequency changes for both megabase- and kilobase-range variants. Megabase-range aberrations were found in 3.4% (9 of 264) of subjects >60 years old; these subjects included 78 MZ twin pairs and 108 single-born individuals from Uppsala ULSAM-cohort. No such findings were observed in 81 MZ pairs or 180 single-born subjects who were <55 years old. Recurrent region- and gene-specific mutations, mostly deletions, were observed. Longitudinal analyses of 43 subjects whose data were collected 7–19 years apart suggest considerable variation in the rate of accumulation of clones carrying structural changes.

Furthermore, the longitudinal analysis of individuals with structural aberrations suggests that there is a natural self-removal of aberrant cell clones from peripheral blood. In three healthy subjects, we detected somatic aberrations characteristic of patients with myelodysplastic syndrome. The recurrent rearrangements uncovered here are candidates for common age-related defects in human blood cells. We anticipate that extension of these results will allow determination of the genetic age of different somatic-cell lineages and estimation of possible individual differences between genetic and chronological age. Our work might also help to explain the cause of an age-related reduction in the number of cell clones in the blood; such a reduction is one of the hallmarks of immunosenescence.

Novel biomarkers for breast cancer; disease prediction and progression

Lars A. Forsberg, Chiara Rasi, Hanna Davies, Jan Dumanski

In collaboration with: Wojciech Zegarski, (Center of Oncology, Bydgoszcz, Poland), Jaroslaw Skokowski, Arkadiusz Piotrowski (Medical University of Gdansk, Poland), Janusz Rys (Jagiellonian University, Krakow, Poland), Tibor Tot (Central Hospital of Falun, Sweden), and Devin Absher (HudsonAlpha Institute, USA)

There exists a paradox in cancer research: although the high mortality from cancer is caused by metastatic spread of tumors, genetic research of metastases is underdeveloped. Contrary to the numerous transcriptome and genome analyses of primary tumors, there is a lack of comprehensive and high-resolution studies comparing genomic profiles of primary tumors and the metastases from the same patient. We have recently completed pilot breast- and ovarian-cancer projects, testing the hypothesis that, upon high-resolution analysis, there are frequent genetic differences between matched primary tumors and lymph node metastases. We observed aberrations that can be linked to metastatic disease and many of the observed differences were previously linked to poor patient survival, based on extensive analyses of

primary tumors. This provides a proof of concept that this approach towards finding new biomarkers for breast cancer progression and patient's prognosis is viable.

The second part of this project deals with search for somatic genetic events in normal breast tissue predisposing to breast cancer. Our previous discoveries of genetic differences between differentiated tissues and in monozygotic twins indicate that the somatic mosaicism for CNVs, between normal cells in the same person is underestimated. This represents a paradigm shift in somatic cell genetics, which has implications for cancer research, as cancer is predominantly a genetic disorder of somatic cells. Hence, this gives an opportunity for analysis of *de novo* somatic aberrations that may predispose normal cells to cancer development, by comparisons of CNV/CpG methylome profiles between an uninvolved margin of histopathologically normal cells surrounding a primary tumor and blood of the same patient.

We compare genomes and epigenomes (CpG methylome) of primary tumors and metastases from patients with breast cancer. We also evaluate genetic and epigenetic (CpG methylation) profiles of normal margin of tissue surrounding primary tumor and blood DNA from the same patient. The objective is to identify patterns suggesting genomic global CNV/epigenetic instability, alternatively aberrations in specific genomic loci that might be coupled to breast cancer progression and predisposition/susceptibility.

Group members during 2015

Jan Dumanski, professor, group leader
Lars Forsberg, researcher, assistant professor
Hanna Davies, research engineer
Dina Mansour Aly, project degree student
Chiara Rasi, research assistant
Edyta Rychlicka, post doc

Funding during 2015

Swedish Research Council, 900 kSEK
Swedish Cancer Society, 1 000 kSEK
Olle Enqvist Byggmästare Foundation, 1 050 kSEK (to L. Forsberg)
Vleugel Foundation, 280 kSEK (to L. Forsberg)

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Genetic Variation and Gene Expression in Human Disease

Lars Feuk

The aim of our research is to understand the importance of genetic variation in the human genome and its role in disease and evolution**.** We are using high throughput sequencing combined with bioinformatic analyses to characterize genetic variation and its correlation with functional data and disease outcomes. The research ranges from very basic studies of genetic variation and transcription to disease specific analysis.

Studies of human disease are mainly focused on neurodevelopmental disorders, including intellectual disability and schizophrenia. We aim to capitalize on the development of the latest sequencing technologies to identify new causative mutations. Our samples include large pedigrees, parent-offspring trios and patient tissue samples, and we are using different analysis strategies to mine DNA and RNA sequencing data for potential causative mutations.

We are also interested in better understanding the process of transcription and RNA processing in human cells. Using data from RNA sequencing, we are aiming characterize splicing mechanisms and investigate the subcellular localization of different transcripts.

Whole genome sequencing of patients with neurodevelopmental disorders

Jonatan Halvardson, Jin Zhao, Eva Carlström, Lars Feuk

To sequence all the coding regions of a genome in a single experiment is a powerful tool to discover disease genes. In this project, we are mainly focusing on two groups of patients to identify causative mutations. First, in collaboration with the clinical genetics unit, we are investigating patients with severe intellectual disability for de novo mutations by whole genome sequencing of both parents and the patient. The second approach is to use pedigrees with multiple affected individuals to identify mutations in regions of linkage or shared homozygosity. Significant work has been invested into establishing a bioinformatics pipeline for analysis of exome and whole genome sequence data.

Functional characterization of mutations causing intellectual disability

Ammar Zaghlool, Jin Zhao, Mitra Etemadikhah, Lars Feuk

Our exome sequencing projects have led to the discovery of several mutations in genes not previously linked to disease. The most interesting genes have been selected for functional follow-up in order to clarify the role of the gene and the specific mutation in disease. Classic molecular biology (cloning, transfection, RNAi) approaches are combined with high throughput genomics such as RNA-seq and CHiP-seq to characterize the role of the genes and mutations.

Transcriptome analysis

Ammar Zaghlool, Jonatan Halvardson, Mitra Etemadikhah, Adnan Niazi, Eva Carlström, Lars Feuk

Transcriptome sequencing is providing novel insights into the transcriptional landscape of cells and tissues. In this project, we use RNA sequencing to study transcription in human tissue samples. In one project, we are investigating subcellular fractions of RNA in order to characterize specific transcripts that are overrepresented in the nucleus or the cytosol of the cell. In another project, we are investigating gene expression in brain samples from patients with schizophrenia and compare expression profiles with tissue from healthy controls.

Group members during 2015

Lars Feuk, senior lecturer, group leader
Mitra Etemadikhah, research assistant
Eva Lindholm Carlström, researcher
Jonatan Halvardson, PhD student
Adnan Niazi, post doc
Ammar Zaghlool, post doc
Jin Zhao, PhD student

Funding during 2015

ERC, 2 556 kSEK
Swedish Research Council, 1 500 kSEK (Medicine and Health), 800 kSEK (Natural and Engineering Sciences

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Human Genomics and Molecular Epidemiology

Ulf Gyllensten

The research of the group is divided into two parts. The first project uses a systems biology approach to study human physiology and in particular the proteome variability. We are interested in how the genetic, epigenetic and environmental factors (medical history, diet, lifestyle) affect the proteome?

Our second project concerns the genetics and clinical epidemiology of cervical cancer. The research focus on the epidemiology of HPV, the identification of genetic factors contributing to the susceptibility and on the interactions between the virus and host susceptibility factors. We have also conducted have a large study comparing cytology (PAP smear) and self-sampling for HPV testing in primary screening to detect women at risk of developing cervical cancer.

Systems biology approach to human physiology

Stefan Enroth, Åsa Johansson, Ulf Gyllensten

We are studying the biological variation in human populations at the level of the genome, transcriptome, epigenome, and proteome. The variation is studied in pedigree-based population cohorts, with unique genetic backgrounds and life style, from the European Special Population Research Network (EUROSPAN). The information includes full exome sequences of selected individuals and imputed exome structure for the complete population, genome-wide analyses of epigenomic state (methylation), high-resolution studies of the plasma proteome, the glycome (glycans), the lipidome, and exposure variables such as medical history, lifestyle and diet.

These multidisciplinary data is used to model the interaction between different types of biological information and address questions that have been beyond the reach for a single discipline. What is the impact of genetic and genomic variation on the plasma proteome? How can genetic, epigenetic, medical history, diet and lifestyle effects be modelled on the proteome? It also represents the first study to integrate data from these multiple layers of biological information and model their interactions and effect on human physiology.

Identification of genetic risk factors for cervical cancer

Dan Chen, Ivana Juko Pecirep, Tao Cui, Stefan Enroth, Ulf Gyllensten

In collaboration with Emma Ivansson (UU)

Cervical cancer is caused by human papillomavirus (HPV) and both genetic and environmental risk factors contribute to persistence of an HPV infection and progression to cervical carcinoma. We have established population-based affected sib-pair (ASP) and case-control cohorts, including over 2,800 cases with cervical carcinoma and 2,000 controls to be used in the identification of genetic risk factors for cervical cancer. This represent the largest set of families with cervical carcinoma identified in the world and among the largest materials for case-control studies.

We have recently performed the first genome-wide association study (GWAS) for this disease, and this has lead to the identification of pathways and individual genes associated with susceptibility to cervical cancer. We now continue with detailed genetic and functional studies of the identified pathways and genes. This project will increase our understanding of the etiology of cervical carcinoma and provide new means for development of diagnostic and therapeutic measures.

Development of rapid and high-resolution methods for HPV typing, and their application to clinical screening of pre-stages for cervical cancer

Inger Gustavsson, Ulf Gyllensten

In collaboration with Matts Olovsson (UU)

We have developed techniques for collection of cervical smear samples (using FTA cards) and detection and quantification of HPV using real-time PCR. These methods allow for detection of individual HPV types and estimation of their titer. The method is economical, easily scalable and amendable to automation, making it suitable for use in primary and secondary screening for cervical cancer pre-stages. We are conducting studies using self-sampling and repeat-HPV typing to determine if this could be used as a strategy in the primary screening for cervical cancer as an alternative to cytology-based strategies.

Evaluation of the use of self-sampling and repeated HPV testing in primary screening for cervical cancer: a randomised study

Inger Gustavsson, Julia Hedlund Lindberg, Pernilla Quarfordt , Ulf Gyllensten

In collaboration with Karin Sanner, Matts Olovsson, Ingrid Wikström, Erik Wilander Riina Aarnio (UU)

The organised gynaecological screening program in Sweden has reduced the incidence of cervical cancer by 50 %. To further reduce the incidence of cervical cancer, the sensitivity of the diagnostic test and coverage of screening must be improved. This can be achieved by introducing human papillomavirus (HPV) typing as the primary diagnostic test and implementing a screening system where women take the samples at their own convenience (by themselves and at home) and send it in to the lab for analysis. The aim is this project is to study: A. The feasibility of using self-sampling at home for HPV testing, as an alternative to collection of samples at a mid-wife's clinic. B. The use of repeated testing for oncogenic forms of HPV as the primary screening test for early detection of cervical cancer. C. The health-economic benefits of using self-sampling and repeated HPV testing as a basis for cervical cancer screening.

Identification of protein biomarkers for identification of with women with HPV infections that may lead to development of cervical cancer

Malin Berggrund, Stefan Enroth, Ulf Gyllensten

HPV is a prevalent virus and most infections are transient. However, a fraction of th HPV infections become chronic and are at high risk of leading to cervical cancer. In this project we are searching for protein biomarkers that could be used to identify women with a chronic infection and early stages of tumor development. The project is based on screening of candidate proteins using the OLINK PEA assay and multiple panels. Such biomarkters could be used in the followup or triage testing of HPV positive women.

Group members during 2015

Ulf Gyllensten, professor, group leader
Malin Berggrund, PhD student
Stefan Enroth, researcher
Inger Gustavsson, research engineer
Julia Hedlund Lindberg, research engineer
Ivana Juko Pecirep, PhD student
Ann-Sofi Strand, lab technician

Dissertations during 2015

Ivana Juko Pecirep, Analysis of genetic susceptibility to cervical cancer using candidate gene and GWAS approaches. May 28, 2015

Funding during 2015

Swedish Research Council, 1 000 kSEK
Swedish Cancer Society, 1 500 kSEK
Swedish Foundation for Strategic Research, 2000 kSEK

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Interplay Between Genetic, Epigenetic and Environmental Factors in the Pathogenesis of Human Disease

Åsa Johansson

In my research I use genome-wide approaches to study how epigenetic and genetic factors interact with the environment in the development of complex diseases. Through large-scale epidemiological and genome-wide association studies we have previously contributed to the identification of many dietary, lifestyle and genetic factors that influence our health and risk for disease.

Recently, we have also shown that various factors, such as genetic variants, chronological age and smoking, affect our genes through epigenetic alterations. Epigenetic changes are heritable from cell to cell, and can therefore persist in the body throughout a lifetime and influence the risk of disease later in life. However, to what extent it can be transmitted through the germ cells and thereby affect our next generation is still an area for debate.

The aim of my research is to determine how genetic and epigenetic factors influence human phenotypes, clinical variables and risk of disease. I also study how environmental factors introduce epigenetic alterations, with subsequent long-term health related effects.

Effects of diet and lifestyle on the epigenome

Weronica Ek, Åsa Johansson

We are investigating the effect of food items, diet and lifestyle on DNA methylation. We are using food frequency questionnaire data together with self-reported lifestyle and clinical variables, which enable us to study epigenetic alterations due to a diet high in e.g. carbohydrates, proteins, or fat, or due to lifestyle factors such as smoking, coffee and alcohol consumption.

Relative contribution of genetic and epigenetic factors in regulating gene expression of disease-related protein biomarker

Muhammad Ahsan, Allan Lind-Thomsen, Weronica Ek, Åsa Johansson

We have recently measured over 150 disease-related protein biomarkers in over 1000 participants of a population based study cohort. By performing genome-wide association study (GWAS) and epigenome-wide association study (EWAS) for each biomarker, we have identified SNPs and CpG methylation that are associated with gene regulation. Integrating GWAS and EWAS data gives a unique possibility to study their respective roles in regulating protein expression. This knowledge is important in order to better understand the role of protein biomarkers in the pathogenesis of human disease.

Infer the causal relation between epigenetic alterations, protein biomarkers and risk of disease.

Muhammad Ahsan, Allan Lind-Thomsen, Åsa Johansson

Protein and epigenetic biomarkers have been identified for many human diseases. A biomarker is increased in patients with a disease, but the direct causal effect of increased levels of most biomarkers has not been widely investigated. Mendelian randomization can be used to evaluate the causal effect of the biomarkers on disease risk and progress. In a Mendelian randomization study, a genetic variant, that increases the levels of a biomarker, is used to divide a population into genotypic subgroups, in an analogous way to how participants are divided into arms in a randomized clinical trial. The aim of this project is to

use Mendelian randomization to evaluate the causal effect of biomarkers on risk of disease, and on disease progress.

Identify epigenetic changes in relation to cardiovascular diseases

Mathias Rask-Andersen, David Martinsson, Muhammad Ahsan, Åsa Johansson

Cardiovascular disease (CVD) is among the leading causes of death worldwide. There are several known genetic and lifestyle risk factors, but association between epigenetics and CVD is poorly understood. We are investigating the link between DNA methylation and CVD. We have performed a genome-wide DNA methylation study in a population-based cohort. Participants were not ascertained upon disease background, but some had a history of CVD, including 48 participants with a previous myocardial infarction. The genes identified are good candidates for additional studies to further understand the pathogenesis of CVD

Group members during 2015

Åsa Johnsson, researcher, group leader
Muhammad Ashan, post doc
Weronica Ek, post doc
Allan Lind-Thomsen, post doc
Mathias Rask-Andersen, post doc
David Martinsson, student
Daniella Ramsey, student

Funding during 2015

SSMF, 944 kSEK
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Mechanisms of adenovirus infection

Ulf Pettersson

Many important discoveries have been made using human adenovirus as an experimental model for control of gene expression. Adenoviruses have moreover become of great interest as gene delivery vectors in gene therapy and as oncolytic viruses in cancer treatment.

How adenoviruses take over the control of host gene expression in infected cells

Ulf Pettersson and Hongxing Zhao

The aim of our project is a detailed characterization of the transcriptome of the virus and that of the infected cell. For these studies we are using state-of-the-art cDNA sequencing technologies.

Our results demonstrate that the adenovirus transcriptome is immensely more complex than hitherto believed with many novel splice sites. An adenovirus landmark map, showing splice and polyadenylation sites, has been constructed. The cellular genes that are up- and down regulated during the course of infection have been identified. In addition, we have identified a set of micro RNAs, which are dysregulated during an adenovirus infection. Our studies of changes in expression of so called long noncoding RNAs have resulted in some unexpected finding. Gradually we are building up a map of the regulatory networks that operate during the different phases of the adenovirus infection.

Epigenetic mechanisms in the human parasite *Trypanosoma cruzi*

Lena Åslund

Some of the major human parasitic diseases are caused by trypanosomes, against which no vaccine and only a few drugs are available. The *Trypanosoma cruzi* genome project has increased our understanding of the genetic make-up of the parasite causing Chagas' disease and will reveal new drug targets, however, several fundamental cellular processes such as transcription and DNA replication are still rather unexplored in these ancient pathogens. We have recently shown that epigenetic signatures, such as acetylated histones H3/H4 and H3K4me3 are associated with transcription start sites in *T. cruzi*, demonstrating for the first time that the 'histone code' is conserved in these protozoan parasites and in polycistronic transcription. We are further investigating the histone modifications during development of the parasite, *i.e*. the replicative insect stage and the non-replicative blood stage in mammalian hosts.

DNA methylation is important in several epigenetic regulations such as gene silencing, cellular differentiation and DNA replication. We have determined the genome-wide distribution of DNA methylation in the *T. cruzi* genome by deep parallel sequencing of immunoprecipitated methylated DNA (MeDIP-Seq). In addition, some of the enzymes involved in this modification are investigated. Further investigations of the function of DNA methylation in trypanosomes will reveal its possible role in the parasite. Elucidating epigenetic mechanisms in the parasite will reveal new approaches to therapies against trypanosomiasis.

Group members during 2015

Ulf Pettersson, professor, group leader
Hongxing Zhao, researcher
Lena Åslund, senior lecturer

Publications 2013-2015

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Genomic Analysis of Gene Regulation

Claes Wadelius

The principles for how genes are activated and inactivated are known but from a genomic perspective our knowledge is very limited. Each cell type has a unique set of active genes that are regulated by the action of a collection of the 2000 transcription factors and other nuclear proteins that bind the DNA molecule. Until recently this could only be studied *in vitro* and for parts of genes. We use chromatin immunoprecipitation (ChIP) to study this *in vivo*. For detection we have developed efficient massive parallel sequencing (ChIP-seq) techniques, which allows us to interrogate the whole genome.

The traditional view of a gene, with a single beginning and end, has been challenged and in addition to the previously known enhancers and other distant regulatory elements, multiple promoters and complex alternative splicing has been found. We therefore annotate all identified DNA-protein interactions relative to everything that is known about the genome. These studies generate massive amounts of data and in order to fully explore the information we develop new informatics strategies and collaborate with specialists in the field. The methods can be used to reveal the mechanisms for common diseases and cancer. We have started to explore this in liver cells and immune cells and have found hundreds of regulatory variants that likely explain association to common metabolic and autoimmune diseases. We have also characterized a large collection of regulatory variants that are excellent candidates to contribute to cancer.

Gene regulatory variants in metabolic and autoimmune diseases and in cancer

Gang Pan, Marco Cavalli, Helena Nord, Madhusudhan Reddy Bysani, Emelie Wallén Arzt

In collaboration with Kerstin Lindblad Toh, Lars Rönnblom and their groups, (UU).

At promoters, enhancers and other gene regulatory elements, nucleosomes are replaced by transcription factors and other regulatory proteins. We map transcription factors to the bases they interact with DNA and in case the cell differs in genetic make up at one base pair, we can tell a difference between what happens at one variant and the other.

Some genetic variants predispose to common diseases and we have started a process to translate this information to molecular mechanisms of disease, primarily for metabolic and autoimmune diseases. We read chromatin signals in relevant tissues to find candidate regulatory elements and test polymorphic variants in cell-based expression systems. The regulatory elements are activated by over-expression of transcription factors that bind to them or by stimulation of primary human cells.

By layering additional large-scale in-house information we have detected thousands of SNPs that are likely to be functional. So far we have detected >100 functional SNPs that are associated to common diseases and intermediary phenotypes and in some cases the molecules that bind differentially between alleles. We have started to assay them using a newly developed high-throughput system.

In one project we investigate the profiles metabolites in the tissues of central importance for diabetes namely pancreatic islets, liver, muscle, fat and also in serum. We do his in all tissues from donors who have type 2 diabetes, prediabetes and who have a normal metabolism. The aim is to find new biomarkers of disease, which may aid the development of new therapies. In addition, we investigate variable gene regulatory signals in key metabolic tissues as a way to find additional variants that predispose to diabetes and related diseases.

In collaboration with Susanne Bornelöv, Umer Husen, Klev Diamanti, Jan Komorowski (UU)

In the cell histone molecules and 147 base pairs of DNA form nucleosomes and many of them have defined positions over genes and around gene regulatory elements. Some histones have epigenetic marks reflecting the function of the specific genomic region and we map these features at the theoretical resolution. We have found that nucleosomes are positioned over exons and have epigenetic marks that are associated to splicing. Other nucleosomes flank gene regulatory elements and carry other epigenetic marks. We have found that nucleosomes at promoters carry specific modifications if they are located in transcribed sequences.

Cancer develops when cells acquire mutations that were not present in the person at birth. In a new project we have started to search for mutations in regulatory elements that contribute to cancer and have a large collection of candidates. The initial experimental validation has shown the expected results. This project is likely to add a new dimension to cancer etiology.

Group members during 2015

Claes Wadelius, professor, group leader
Marco Cavalli, researcher
Gan Pan, researcher

Funding during 2015

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Publications 2013-2015

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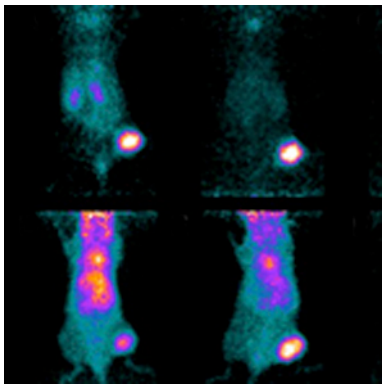
Medical Radiation Sciences



Ionizing radiation is widely used in medicine for diagnostics and therapy of different diseases. Radionuclide imaging facilitates detection of the disease-associated molecular phenotype of tissues, and selection of optimal therapy. External beam radiation therapy is an efficient way to treat localized cancer by a concentrated dose to the tumour, while targeted delivery of cytotoxic radionuclides may be efficient for eradiation of disseminated cancer. The use of radioactive tracers *in vitro* and *in vivo* can also elucidate many aspects of normal biology and pathogenic alterations in biochemistry.

The aim of our research is to widen the knowledge permitting the use of radiation for medicine and in basic biology. The programme includes research in basic radiation biology to understand how living cells respond to radiation, optimal methods for radiation treatments, applied dosimetry, and development of phenotype-specific delivery of diagnostic and therapeutic radionuclides to malignant tumour cells.

In several projects we collaborate with the clinics for nuclear medicine, oncology and medical physics at the Uppsala University hospital, as well as with many researches in Sweden and abroad

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Medical Radiation Physics

Anders Ahnesjö

Our research focuses on the application of physics and engineering concepts to radiation in medicine, with a specific emphasis on radiotherapy where we try to find methods that can increase cure and reduce side effects. We have a multiscale approach ranging from investigations at the nanometer range where we study clustering effects of ionization events, to the centimetre range where we study interference of patient movements with radiation beam patterns. Of particular interest is the use of protons and other light ion beams as these can deliver therapeutic doses to a tumour with reduced dose burdens to healthy tissues as compared to the commonly used treatments with photon beams.

Track structure based biological effectiveness analysis and modelling

Villegas-Navarro, Gloria Bäckström, Nina Tilly and Anders Ahnesjö

Different radiation modalities such as low energy photons, proton beams or carbon ion beams, have different variation in biological response per dose. We use a Monte Carlo track structure code, LIonTrack, to simulate the energy deposition around particle tracks in an event-by-event mode for different radiation qualities such as photons, protons and other light ions. Analyses of the clustering pattern of the energy deposition sites on a nanometric, biomolecule scale indicate that cluster type characterization correlates better to biological response effectiveness than the macroscopic quantity linear energy transfer (LET) commonly used as radiation quality descriptor. Hence, clustering properties may add valuable information for a more detailed prediction of the variation of the biological response effectiveness, and

eventually model the variations in treatment planning of proton therapy to allow for a more optimal utilization of its merits.

Interplay effects of scanned proton beams with patient movement

David Boersma, Ulf Isacsson, Anders Montelius, Hediye Acun and Anders Ahnesjö

In collaboration with Kristina Nilsson

Modern proton therapy facilities apply a technique where a narrow beam is scanned over the tumour volume to be treated. A risk factor with scanned proton radiation is that patient movements during irradiation may interact with the scanning movement of the beams. These interplay effects may result in that parts of the tumour receive less than the planned dose, or parts of a nearby organ at risk gets overdosed.

In this project we develop a computer based simulation environment for detailed study of the processes to aid in quality assurance of proton treatments. Based on the radiation transport code packages Geant4 and GATE, and dose accumulation software using CT images for different phases of patient movements detailed studies will be enabled. This may prove particular important for treatment of childhood cancers since children have long expected life time after treatment, and to minimize risks for late side effects the best possible radiation technique must be used, to which this project hopefully will contribute.

Application of optical body surface scanning in the thorax region

Kenneth Wikström, Ulf Isacsson and Anders Ahnesjö

In collaboration with Kristina Nilsson

A problem common for several radiotherapy scenarios is to establish the accuracy and precision with which practical motion indicators can be used for in-beam tumour positioning or out-of-beam protection of organ at risk. Photogrammetric methods using optical scanning of the body contour is a promising method, which is commercially available. We aim to investigate and develop the clinical applicability of such data in particular for two patient groups: left sided breast cancer where heart protection is crucial for prevention of heart complications later in life, and lung cancer as to precisely hit the tumour.

Rectal wall protection and in vivo dose determination with a rod retractor

Andreas Johansson, Ulf Isacsson and Anders Ahnesjö

In collaboration with Gunilla Ljung, Kristina Nilsson

Due to the proximity of the prostate to the large bowel there is risk for rectal side effects in radiotherapy of prostate cancer. The distance between the prostate gland and the rectal wall can be increased by means of a rod retractor that pushes the rectum backwards during treatment. The rectal wall can then be moved out from the high dose region close to the prostate. Also, due to the induced tension of connective tissues it is hypothesized that the prostate gland is immobilized so that smaller margins can be used while aiming beams at the prostate as to further reduce healthy tissue dose burdens. The study is implemented as a collaboration between Mälarsjukhuset Eskilstuna and Uppsala University Hospital.

Probabilistic evaluation and optimization of radiotherapy treatment plans

David Tilly and Anders Ahnesjö

Uncertainties in radiotherapy delivery are routinely handled by expanding the target volume with a standardized margin to ensure adequate dose coverage. An alternative is to employ a probabilistic based planning procedure where patient specific uncertainties are explicitly considered to find the best treatment plant. This can be very computational intensive needing

several hundred simulations per patient of the interplay between anatomy and the radiation beam to sample the involved uncertainties. The project aims to find efficient calculation methods as to make the approach practical for clinical applications.

Dose Painting - use of functional imaging for radiotherapy dose prescriptions

Eric Grönlund, Anders Montelius and Anders Ahnesjö

In collaboration with Silvia Johansson, David Kudrén and Mark Lubberink (UU)

In routine radiotherapy the dose prescription is given as a certain dose level to be given for the entire tumor target volume usually defined on anatomical CT images. Functional imaging can potentially be used to prescribe a heterogeneous dose distribution, "dose painting", tailored to achieve equal tumor control probability with a smaller amount of radiation. We retrospectively study patient groups with known given doses and probabilities for cure as to correlate with data from pre-treatment functional imaging. The goal is to develop the mathematical formalism and establish data for optimization of dose painting in clinical practice for design of prospective clinical studies.

Radiation safety strategies in diagnostic radiology

Hans-Erik Källman and Anders Ahnesjö

Radiation used in diagnostic imaging is one of the largest dose contributors to humans from artificial sources. Image metadata is a systematic source of information containing useful exposure indicators. In this project, image metadata has been proved useful for dose management. Retrospective metadata, together with images from computed tomography examinations in the county of Dalarna are now used as input to Monte Carlo simulations for a more detailed analysis of patient dose distributions and organ doses. This will form a basis for improvement of dose management strategies for reduction of dose exposures on a population level.

Group members during 2015

Anders Ahnesjö, professor, group leader
Hediye Acun, visiting researcher
David Boersma, researcher
Gloria Bäckström, visiting researcher
Erik Grusell, associate professor, hospital physicist
Eric Grönlund, PhD student
Ulf Isacsson, PhD, hospital physicist
Andreas Johansson, hospital physicist, PhD student (Eskilstuna)
Hans-Erik Källman, hospital physicist, PhD student (Falun)
Anders Montelius, associate professor, hospital physicist
Tufve Nyholm, associate professor, hospital physicist
Carl Sjöberg, industrial PhD student
David Tilly, industrial PhD student
Nina Tilly, associate professor, industrial affiliate
Fernanda Villegas Navarro, PhD student
Kenneth Wikström, hospital physicist, PhD student

Funding during 2015

Swedish Childhood Cancer Foundation, 600 kSEK
Swedish Radiation Safety Authority, 500 kSEK
Stiftelsen Onkologiska klinikens i Uppsala Forskningsfond, 200 kSEK
ALF, project grant 165 kSEK
ALF, salary grant 300 kSEK
FoU-centrum Landstinget Sörmland, 300 kSEK, salary PhD student
Centrum för Klinisk Forskning Dalarna, 415 kSEK, salary PhD student

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Head and Neck Tumour Targeting

Marika Nestor

The aim of our research is to find an efficient method for diagnosis and therapy of head and neck cancer. We focus on developing the use of radioactive nuclides for localising and treating metastasised tumours, with the aim to improve the survival of this group of patients.

In Sweden approximately 1000 cases of cancer in the mouth or throat are discovered each year. This is a type of cancer that is relatively difficult to treat since it often spreads to other parts of the body, and around 50 per cent of the patients eventually die from their disease. Radiation and/or surgery are standard therapies for these tumours but these methods are not sufficient for localising or treating metastasised tumour cells today.

Use of radio-immunotargeting to improve diagnostics and therapy of head and neck squamous cell carcinoma

Diana Spiegelberg, Anna-Karin Haylock, Anja C. Mortensen, Jonas Stenberg, Marika Nestor

In this project we aim to improve diagnostics and therapy of head and neck squamous cell carcinoma (HNSCC), by the use of radio-immunotargeting. We identify and characterize different promising antigens as new molecular targets in this setting. Novel tumour-targeting molecules are then developed and evaluated. Finally, the targeting molecules are radio-labelled with various radionuclides, and the radio-conjugates are optimized and assessed for diagnostic and therapeutic potential.

We are assessing the possibility of targeting several promising novel therapeutic targets for radionuclide targeting. We are studying the density and distribution of primarily, but not limited to, different splice variants of CD44. In cases where surface markers differentially expressed in subpopulations are identified, subpopulations are evaluated for differences in e.g. proliferation, migration and radioresistance *in vitro.*

This is a translational project with the established goal of ultimately evaluating the most promising conjugate in the clinic. In the initial stages we mainly focus on developing conjugates for molecular imaging. Currently, we are assessing several different formats targeting CD44v6, such as antibody single-chain variable fragments (scFv), antigen-binding fragments (Fab fragments) and bivalent Fab Mini-antibodies (functionally equivalent to Fab2 fragments), as well as a promising peptide towards CD44v6, and a promising antibody towards EGFRvIII. We also assess different radionuclides and labelling methods in order to form our targeting radioconjugates, and evaluate the binding interactions and cellular processing of the conjugates in tumour cells both *in vitro* and *in vivo*.

Improving cancer therapy by combining radio-immunotherapy and p53 therapy

Anja C. Mortensen, Diana Spiegelberg, Marika Nestor

The main objective for this project is to combine two cancer therapies, radio-immunotherapy and p53 therapy, to improve treatment outcomes and prolong patient survival. Ionizing radiation has been shown to induce p53-dependent *Mdm2* gene transcription, eventually resulting in degradation of p53, leading to prevention of apoptosis. However, blocking the Mdm2/p53 interaction actively prevents this degradation, and could therefore improve the effectiveness of radio-immunotherapy.

Several tumour associated antigens are investigated, and suitable targeting agents towards these targets are then selected. So far, we have focused on EGFRvIII, EGFR and CD44v6 as tumour targets, and antibodies or antibody fragments binding to one of these antigens as the

targeting molecules. Selected molecules are then assessed for radiolabelling of suitable therapeutic radionuclides. We use radionuclides of interest for therapy, such as ^{177}Lu and ^{131}I, but we are also assessing more diagnostic radionuclides such as ^{111}In, in case we obtain high synergistic effects with the combination therapies.

Cytotoxicity of peptides targeting the MdM2/p53 interaction is assessed in order to find suitable concentrations for combination therapy. Cytotoxicity of selected radio-conjugates of different specific activity is assessed in the same way. Finally, the cytotoxicity of p53 therapy, radio-immunotherapy, and the combination of the two in monolayer cell assays (where applicable) and in tumour spheroids is assessed.

For the most suitable combinations of radio-conjugates and p53 peptides, we plan to move on to therapy studies in tumour bearing mice. The optimal doses and specific activity for peptides and radio-conjugates will be evaluated, as well as i*n vivo* kinetics, tumour uptake and uptake in normal tissue. Therapy experiments, in which mice will receive a) no treatment, b) p53 therapy, c) radio-immunotherapy, and d) p53 therapy and radio-immunotherapy, will then be performed.

Tools for the characterization of heterogeneous protein interactions

Hanna Björkelund, Sina Bondza, Jos Buijs, Jonas Stenberg, Karl Andersson

Proteins are biological macromolecules that are essential for life. They serve as structural components in the cells and are involved in almost all biological processes. Their function can be catalytical (enzymes), DNA triggering (transcription factors) or involved in the immune response (antibodies), to mention a few possibilities. In most cases, proteins typically interact with other molecules and proteins in order to perform their tasks. The characterization of protein interactions is therefore an important part of cell-biology research.

The aim of the project is to improve the tools for characterization of protein – cell interactions, both from a measurement point of view and an analysis point of view. We have developed a novel class of biosensor that is capable of detecting how proteins bind to cells in real-time, and are now focusing on data analysis tools for interpretation of the acquired binding traces.

The majority of current biomolecular interaction analysis is based on simple models and assumptions, like 1:1 interactions ($L + T \leftrightarrow LT$). The heterogeneous cell surface contradicts such assumptions, and we therefore believe that a better description of protein cell interactions can lead to important improvements of how biological processes are explained and understood.

Group members during 2015

Marika Nestor, researcher, group leader
Karl Andersson, adjunct senior lecturer
Sina Bondza, degree project worker
Jos Buijs, guest researcher
Hanna Björkelund, guest researcher
Anna-Karin Haylock, PhD student
Anja Mortensen, PhD student
Emma Rodrigues, degree project worker
Diana Spiegelberg, PhD student
Jonas Stenberg, Industry PhD student

Dissertations during 2015

Diana Spiegelberg, Towards Personalized Cancer Therapy: New Diagnostic Biomarkers and Radiosensitization Strategies, May 13, 2015.

Funding during 2015

Swedish Research Council, 1 090 kSEK
Swedish Radiation Safety Authority, 250 kSEK
Swedish Cancer Society, 500 kSEK
Swedish Society for Medical Research, 700 kSEK

Publications 2013-2015

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Radiation Biology and DNA Repair

Bo Stenerlöw

Cancer therapy with ionizing radiation is lethal to tumour cells because induced DNA double-strand breaks (DSBs) are not correctly repaired. The last decades, research on DNA repair has lead to many novel insights in cellular repair but several important aspects of radiation-induced DSB are still unresolved. For instance, it is still unclear how primary damage is detected, how this initiates signal transduction and activates DNA repair proteins, selection of repair pathway and how DNA repair mechanisms are affected by radiation quality (*i.e.* clustered damaged DNA sites generated by high LET radiation). As we gain a better understanding of DSB repair mechanisms and the regulation of pathway choice, it is likely that basic mechanistic insights will translate into clinical benefits.

Repair pathways and signalling

The main repair pathway of radiation-induced DSBs is non-homologous end-joining (NHEJ). The rapid binding of broken DNA ends is a key event in repair of DSB and cells defective in NHEJ are extremely sensitive to ionizing radiation. The function of this initial step and the following protein interactions may largely affect the outcome of repair. Although the major protein complexes involved in NHEJ have been identified, it is still not fully understood how, when and where the major protein complexes come together and repair DSB.

We are currently investigating how NHEJ proteins interact and how they may regulate other repair pathways and cellular processes.

Clustered damaged sites in DNA

Recent and planned radiation therapy modalities use high-LET (LET: linear energy transfer) radiation, in terms of accelerated ions or radioactive nuclides emitting α-particles or Auger-electrons in order to effectively treat malignant tumours: a relatively low dose of high-LET radiation has a high cell killing efficiency. However, the number generated DSB is similar to that induced by conventional gamma radiation and this strongly implicate that DSB is a highly heterogeneous type of DNA damage: the dense deposition of energy from high-LET radiation results in both complex DSBs (*i.e.* DSBs associated with additional DSBs, SSBs or base lesions within 20-30 bp) and clustered DNA breaks within 1-2 Mbp of chromatin. It is evident that clustered lesions are much more difficult to restore, but there is no information about failure in specific steps in the repair process.

Our research is focused on DNA damage localization within chromatin and the mechanisms involved in DNA damage recognition at clustered damaged sites.

Sensitizing tumour cells to radiation

New knowledge about DNA repair mechanisms and how these are affected by radiation quality and targeting of growth factor receptors commonly overexpressed in tumour cells, have the potential to further increase the efficacy of radiation treatment of tumours. Importantly, even a relatively small modification of the radiation response in the tumour cell population may have a significant impact on the probability to kill all clonogenic tumour cells over several weeks of IR fractionation or radionuclide exposure.

Several drugs are known to sensitize cells to IR and considering the potential lethal induction of DNA double-strand breaks, drugs that interfere with the repair of these breaks are obvious candidates. In recent years, there has been rapid progress in the identification of new molecular targets that could be useful for cancer therapies. Some of these promising

targets are members of the heat shock protein (HSP) family, which is a group of proteins that are induced in response to cellular stress.

We here investigate novel HSP90 inhibitors by characterizing their cellular and molecular effects, and their effects on cells and tumours in combination with IR.

Group members during 2015

Bo Stenerlöw, professor, group leader
Andris Abramenkovs, PhD student
Sara Ahlgren, researcher
Christina Atterby, research engineer
Amelie Fondell, researcher
Lars Gedda, adjunct professor
Ann-Sofie Gustafsson, PhD student
Diana Spiegelberg, PhD student

Dissertations during 2015

Ann-Sofie Gustafsson, Radiation response in human cells. DNA damage formation, repair and signaling, December 16, 2015.

Funding during 2015

Swedish Cancer Society, 600 kSEK (B Stenerlöw), 400 kSEK (L Gedda)
Swedish Radiation Safety Authority, 500 kSEK

Publications 2013-2015

1. Guidi R, Guerra L, Levi L, Stenerlöw B, Fox JG. Josenhans C, Mascucci MG, Frisan, T. Chronic exposure to the cytolethal distending toxins of Gram-negative bacteria promotes genomic instability and altered DNA damage response. Cell Microbiol. 2013, 15(1):98-113.
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Scaffold Protein-Based Radionuclide Tumour Targeting

Vladimir Tolmachev

Therapy of disseminated cancer can be improved by increasing treatment specificity with the use of molecular recognition of proteins that are aberrantly expressed in malignant cells. Antibodies, tyrosine kinase inhibitors and small interfering RNAs are just a few examples of novel specific therapeutics. However, the expression of a particular molecular target can vary from patient to patient and between lesions within the same patient. Therefore, a molecular testing is becoming to be a part of the paradigm of targeted therapy to choose drugs on an individual patient basis.

Radionuclide molecular imaging of tumour-associated targets has the clear advantages of being global, minimally-invasive and easily repeatable to follow changes in a target expression. Therefore, radionuclide molecular imaging might be used for patient stratification identifying patients who would most likely benefit from particular targeting therapy due to sufficient target expression. Thus, radionuclide molecular imaging may be a powerful and convenient tool to make treatment of disseminated cancer more personalised.

Predictive biomarkers identify only high probabilities of response to a targeting therapy. Some patients with positive predictive biomarkers will inevitably not respond. Targeted delivery of cytotoxic nuclides (e.g. beta- or alpha-emitters) may provide selective destruction of malignant cells sparing healthy tissues. The use of radionuclides offers advantage of cross-fire effect (when nuclides delivered to one cancer cell irradiate its malignant neighbours) and absence of multidrug resistance phaenomenon.

New type of targeting probes, scaffold proteins

Javad Garousi, Hadis Honarvar, Joanna Strand, Mohamed Altai, Dan Sandberg, Jörgen Carlsson, Anna Orlova, Joachim Feldwisch, Fredrik Freijd, Vladimir Tolmachev

The use of robust protein scaffolds enables selection of high-affinity binders that are much smaller than antibodies. Our team pioneered in the use of scaffold protein for molecular imaging *in vivo* by radiolabelling of Affibody molecules. Affibody molecules are small (7 kDa) phage-display selected scaffold proteins, developed at Royal Institute of Technology (KTH), Stockholm. They can be selected for specific binding to a large variety of protein targets including tumour-associated antigens. Currently, anti-HER2 Affibody molecules are evaluated in clinical studies demonstrating exquisite sensitivity and specificity. In 2015, we reported successful use of another type of scaffold protein ADAPTs (5.2 kDa), for molecular imaging.

Our group focuses on evaluating the influence of different factors (format, labelling chemistry, off-target interactions) on tumour targeting using scaffold proteins.

Personalising tyrosine kinase targeting

Hadis Honarvar, Javad Garousi, Joanna Strand, Mohamed Altai, Anna Orlova, Fredrik Freijd, Vladimir Tolmachev

Trans-membrane receptor tyrosine kinases (RTKs) are overexpressed in many malignancies. RTK signalling triggers cell proliferation, the suppression of apoptosis, increased motility and the recruitment of neovasculature. Overexpressed RTKs are the molecular targets for an increasing number of anti-cancer drugs.

We focus on the use of radionuclide molecular imaging for personalising of tyrosine kinase treatment. The main targets are HER2, EGFR, HER3, IGF-1R, VEGFR2 and PDGFRβ.

Influence of target expression level in tumours and normal tissues, cellular processing of tracers in tumours and excretory organs, affinity of a tracer on imaging sensitivity is evaluated and used in rational molecular design of scaffold proteins-based probes for RTK imaging.

Affibody-based pretargeting for radionuclide therapy of cancer

Hadis Honarvar, Mohamed Altai, Maria Tsourma, Justin Velletta, Anna Orlova, Vladimir Tolmachev

High reabsorption of radiolabelled scaffold proteins in kidneys makes radionuclide therapy challenging. To avoid this issue, we started development of pretargeting for Affibody-based therapy. Radionuclide pretargeting is a two-step procedure for selective delivering of radionuclides to tumours. In this case, a primary Affibody-based targeting agent fitted with a recognition tag is injected first. After localization of the primary agent in a tumour and its clearance from blood and other non-targeted compartments, a radiolabeled secondary agent, which is specific to a recognition tag, is injected. The secondary agent is selected to have low re-absorption in kidneys.

We have shown feasibility of the use of bioorthogonal chemistry and peptide nucleic acids (PNA) interactions as mechanisms for secondary recognition. Hadis Honarvar received the prestigious Marie Curie Award from European Association for Nuclear medicine for development of MPA-mediated Affibody pretargeting.

Group members during 2015

Vladimir Tolmachev, professor, group leader
Mohamed Altai, post doc
Jörgen Carlsson, professor emeritus
Joachim Feldwisch, visiting researcher
Joanna Strand, PhD student
Fredrik Frejd, adjunct professor
Javad Garousi, PhD student
Hadis Honarvar, PhD student
Anna Orlova, senior lecturer, visiting researcher
Dan Sandberg, PhD student
Justin Velletta, degree project worker
Maria Tsourma, degree project worker

Dissertations during 2015

Joanna Strand, Affibody Molecules for PET Imaging. October 3, 2015

Funding during 2015

Swedish Cancer Society, 800 kSEK
Swedish Research Council, 900 kSEK
Swedish Research Council, 123 kSEK (Personal research position, V. Tolmachev, January-March 2015)
Ankarstrands and Claesons foundations, 264 kSEK (Postdoctoral scholarship, M. Altai)

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6. Strand J, Honarvar H, Perols A, Orlova A, Selvaraju RK, Karlström AE, Tolmachev V. Influence of macrocyclic chelators on the targeting properties of (68)Ga-labeled synthetic affibody molecules: comparison with (111)In-labeled counterparts. PLoS One. 2013, 8(8):e70028.
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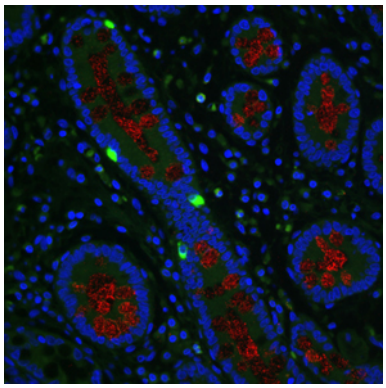
Molecular Tools



Molecular tools for molecular medicine are in rapid development. Radically improved methods can offer entirely new biological insights, reveal disease processes at potentially curable stages, and serve to evaluate new drugs and monitor responses to therapy.

The Molecular Tools unit at the Department of Immunology, Genetics and Pathology has a strong tradition of developing molecular tools to quantify or image the distribution of DNA, RNA, and protein molecules in biological samples such as blood and tissues.

Advanced Molecular Tools in Genomics, Proteomics and Medicine

Ulf Landegren

Our group has pioneered methods such as oligonucleotide ligation assays, padlock, selector, and proximity probes, as well as the novel nFold and ExCirc probes and super rolling circle amplification, currently under development in our lab. We apply these methods together with collaborating partners in a wide range of biomedical analyses with some focus on malignancy, neurodegeneration, cardiovascular disease, autoimmunity and infectious disease. The lab also very actively disseminates our techniques for example by making them available nationwide as services via the Science for Life Laboratory organization, or through licensing leading international biotech and diagnostic companies, or via the so far seven companies we have spun out.

Our molecular probes typically represent little molecular machines with elements for affinity reaction to proteins or nucleic acids, and others susceptible to enzyme catalyzed reactions that serve to enhance specificity of detection or sensitivity of readout.

Our quite general detection procedures permit highly specific solution-phase or localized analyses of large sets of potential biomarkers, extending even to the single-copy level to evaluate molecular heterogeneity among individual cells and throughout tissues, and the techniques are promising for a new generation of high-performance point of care analyses. Some of our ongoing projects are highlighted below.

Super rolling circle amplification and applications for ctDNA mutation detection

Lei Chen

Using a technique I have recently developed it is now possible to locally amplify individual detected nucleic acid or protein molecules with extreme specificity to easily detected levels. The techniques offers radically new opportunities to enhance visualization in situ, obtain digital readout of multiplex biomarker assays, or clone DNA molecules at 100% efficiency and with no need for bacterial transformation. In a longer perspective the technique is promising for demanding detection reactions at the point of care. I am currently using the technique to investigate the presence of tumor-specific mutant DNA in plasma from patients treated for cancer. Using flow cytometric readout we are able to find single mutant DNA

sequeces in the presence of 100,000 wild-type fragments, and we can detect multiple mutations from single patient sample using a multiplex approach.

Molecular tools for analysis of drug binding characteristics

Abdullah Al-Amin

Structural similarities in active sites of drug targets lead to risks of poor selectivity and unwanted side effects in rational drug design. There is a great need for more accurate techniques to monitor selective binding and correct localization of a candidate drug and its target interaction in healthy or pathological clinical specimen in the process of drug discovery in preclinical studies. In a first phase, we have developed very sensitive and specific *in situ* drug-target interaction detection methods TE-MA (Target Engagement–Mediated Amplification), where target binding by DNA-linked kinase inhibitors were visualized and quantified in cells and tissues by rolling-circle amplification (RCA) and Pharma-PLA, using the proximity ligation assays mechanism. The methods serve to investigate selective target binding and correct localization of candidate drug in relevant clinical specimen during lead optimization in preclinical drug discovery. Another on going effort is aimed to combine the cellular thermal shift assay (CETSA) with multiplex proximity extension assays (PEA) for quantitative drug proteins interaction analysis. Preliminarily we have developed CETSA-PEA assay in cell extracts and next aim apply this novel approach in consecutive fresh frozen samples of nucleated blood cells from leukemia patients before and after initiation of targeted therapy.

A platform for sensitive protein detection

Tonge Ebai

There is a great need for protein detection at improved sensitivity, in particular since ultrasensitive protein detection greatly expands the potential ranges for biomarkers, and it may translate to earlier diagnosis of disease processes, which in turn can improve chances for successful treatment outcomes. I am developing protein assay formats that enhance specificity of detection, reduce nonspecific background, and permit strongly amplified detection signals even using standard assay formats and instrumentation readily available in hospitals and research labs. In one approach, PlaRca, proteins are captured from biological samples via antibodies immobilized in microtiter wells. The proteins are then detected via two further antibodies that have been modified with oligonucleotides such that they can template the formation of a circular reporter DNA strand for amplified detection via rolling circle amplification. A variant of this assay takes advantage of reagents already developed for proximity extension assays, but combines these with capture probes that permit analysis of larger sample volumes, removal of extraneous components through washes, and that increase the specificity of recognition, just as in PlaRca, via the need for triple recognition of target molecules. I demonstrate the added value of these assay formats by exploring analyses of clinically relevant, but weakly expressed biomarkers.

Single cell proteomics

Caroline Gallant

The ability to investigate biological phenomena at the level of single cells is attracting increasing interest as a means to characterize cellular heterogeneity and to explain biological responses by individual cells. While recent years have seen great progress in such analyses at levels of transcripts, deeper understanding of functional differences among single cells, effects of malignant transformation, and responses to targeted therapies will necessitate the ability to monitor in individual cells both RNA and protein molecules in parallel. We are

developing and applying protein assays (e.g. proximity extension assays) in parallel to RNA detection for multi-parameter characterization of single cells, enabling parallel analyses of unprecedented numbers of proteins per individual cell. Analytical tools that we developed are being offered as services for Swedish scientists via the SciLifeLab Single Cell Proteomics Facility.

Precise mapping of cell signaling pathways in cells and tissues

Peter Lönn

In this project I am optimizing both PLA and PEA to measure large numbers of proteins, post-translational modifications, and protein-protein interactions in parallel in fixed cells or tissues. The goal of the project is to develop methods to screen biomarkers or to examine complex signatures of protein and modifications to better define cellular states and responses. In addition, I also combine these molecular tools with classical biochemical assays to precisely map dynamics of cell signaling pathways and to bring new insights about how post-translational modifications and interactions are regulated. The above approaches can greatly improve opportunities to investigate cellular functions in health and disease, and in responses to experimental or established molecularly targeted therapies.

Dried blood spots for easy sample handling and RCA Reporters for simplified and improved RCA based detection assays

Johan Björkesten

Capillary dried spots of blood or plasma, sampled from a finger prick offers many important advantages over venous sampling. These advantages include no need for trained personnel during sampling, no transportation regulation enabling sampling at home, and inexpensive storage of even very large biobanks and routine testing for wellness. A major limitation with dried blood spots is the limited sample amount. Methods developed in our lab (proximity assays for protein detection) consume minute amount of sample and we have demonstrated excellent correlation between wet and dried samples also in highly multiplex protein measurement.

RCA Reporters are new tools, currently under development in our lab, for highly specific and sensitive rolling circle amplification (RCA)-based detections with a single step protocol. Preformed circular RCA templates are added to the sample together with all other necessary components. Only in the presence of specific target molecules does amplification occur that generates an easily detectable signal. The simplicity of RCA Reporters potentially makes them suitable for point of care applications for detection of either nucleic acids or proteins. Another possible implementation of RCA Reporters is to increase the power of any RCA method simply by adding RCA Reporters to the RCA mix. This will drastically reduce incubation times, or increase the size of the original rolling circle products making them large enough to be detected by e.g. regular flow cytometers or perhaps the naked eye.

Molecular tools for sensitive point of care infectious diagnostics

Phathutshedzo Muthelo

The turn-around time of an infectious diagnostic test is an important parameter in controlling disease spread and choosing appropriate treatment regiments. Current molecular methods with quick turn-around times require costly equipment and skilled technicians to operate, making them unsuitable for use in low resource environments where rapid infectious diagnostics are needed. Thus, using molecular tools previously developed in our lab such as ExCirc probes and the Proximity assays, we aim to develop rapid point of care diagnostics systems for infectious diagnosis in low resource settings. ExCirc probes are nucleic acid

amplification probes that require multiple hybridization and enzymatic events to yield circular DNA molecules that can be amplified through rolling circle amplification. These probes offer increased specificity by requiring multiple recognition events while isothermal rolling circle amplification avoids the need for PCR equipment for amplification. Along with developing these probes we aim to use simplex and multiplex readout methods that do not require complex equipment and skilled technicians to analyze.

Proximity assays for proteome analyses and biomarker validation

Masood Kamali-Moghaddam, Radiosa Gallini, Liza Löv, Felipe Oliviera, Qiujin Shen, Lotta Wik, Agata Zieba

Using various proximity assays, specific proteins as well as their interactions and modifications, can be analyzed by translating detection reactions to reporting DNA sequences. In these methods protein-binding reagents are modified by conjugation to DNA oligonucleotides. When two or more of these modified binders recognize a target molecule or a pair of interacting proteins, the free ends of the attached oligonucleotides are brought in proximity and can subsequently be joined by DNA ligation. The ligation products are amplified by PCR enabling sensitive detection. The PLA technique can be carried out in solution – requiring very small amounts of materials to be tested – or on a solid phase whereby the target molecules to be detected can be first immobilized via affinity probes, while other materials are removed by washes. In a yet another format of PLA (*in situ* PLA) that can be used for protein analyses in cells and tissues the oligonucleotides are designed to guide circularization of two accessory linear DNA molecules. The DNA molecules that form by ligation are amplifiable by rolling circle amplification and visualized using epi-fluorescence or confocal microscopes.

The combination of the use of two or more binding reagents and efficient DNA amplification provides high sensitivity and specificity of detection, surpassing conventional protein detection methods. PLA can therefore provide a powerful molecular tool for protein measurements at extremely low concentrations.

We continuously improve methods for sensitive proteome analyses, aiming for further improved sensitivity of detection and for simultaneous detection of proteins in highly multiplexed formats. In addition to sensitive detection of soluble proteins, different variants of the technology has been used to establish assays for detection of immune complexes, aggregated proteins, fusion proteins and micro vesicles. For instance, in the field of neurodegenerative disorders we have developed a sensitive assay for specific detection of protein oligomers that plays a central role in diseases such as Alzheimer, prion and Parkinson diseases. Using *in situ* PLA, we have established extremely specific and sensitive assays to study protein interactions and posttranslational modifications such as phosphorylation of Tau protein, which plays a central role in development of Alzheimer's disease. In addition, we have developed a multiplex PLA in which up to 47 proteins are analyzed simultaneously using very small amount of patient samples. The Multiplex PLA has, for instance, been used to screen blood samples from patients with chronic pain, and cerebrospinal fluid samples from patients with amyotorophic lateral sclerosis, and we have identified several biomarker candidates in the latter disease.

We have also developed a version of PLA (4PLA) in which requirement of simultaneous binding of five different antibodies allows specific detection of more complex target molecules. Using this sensitive assay form we have for the first time been able to detect prostasomes in blood plasma – establishing these as a member of a new class of biomarkers generally referred to as microvesicles/exosomes. 4PLA-based detection of prostasomes revealed elevated levels of these microvesicles in samples from prostate cancer patients, and

the analysis also demonstrated that the concentration of prostasomes better reflects disease aggressiveness than the currently used PSA test.

Currently, we utilize multiplex proximity assays to identify and characterize a large number of classes of microvesicles originating from different organs – such as prostate, lung and breast – in order to establish new sensitive and reliable diagnostic and prognostic tests using this novel class of biomarker candidates.

Using multiplex *in situ* PLA we have established a unique method for multicolor, specific and sensitive detection of microvesicles via flow cytometry, which allows identification of different microvesicles originating from different organs and/or cells in complex matrices such as blood plasma.

The flow cytometry-based PLA has also been used to establish sensitive assays for detection of fusion proteins such as BCR-ABL in chronic myeloid leukemia. Since the detection is carried out in intact cells, the method allows simultaneous immunofluorescence staining in order to identify cell populations that are expressing the fusion protein.

Group members during 2015

Ulf Landegren, professor, group leader
Abdullah Al-Amin, PhD student
Johan Björkesten, PhD student
Lei Chen, PhD student
Marcus Danielsson, research engineer
Tonge Ebai, PhD student
Elin Ekberg, administrative assistant
Caroline Gallant, researcher
Joakim Galli, project coordinator
Johanna Herö, research engineer
Tomas Klingström, guest PhD student
Peter Lönn, researcher
Johan Oelrich, systems developer
Mike Taussig, researcher
Erik Ullerås, project coordinator
Rachel Yuan Nong, post doc

Group member establishing independent research
Masood Kamali Moghaddam, associate professor, group leader
Radiosa Gallini, research engineer
Liza Löf, PhD student
Felipe de Oliviera, PhD student
Qiujin Shen, researcher
Agata Zieba Wicher, researcher
Lotta Wik, researcher

Funding during 2015

Ulf Landegren
Swedish Research Council 4 000 kSEK (Medicine and Health), 980 kSEK (Natural and Engineering Sciences, 1 000 kSEK (Infrastructures)
ERC, 4 500 kSEK
EU, FP7, 850 kSEK
IMI, 2 500 kSEK

Masood Kamali Moghaddam
EU, ITN (GastricGlycoExplorer), 1 800 kSEK
Uppsala Berzelii Technology Centre for Neurodiagnostics, 550 kSEK

Publications 2013-2015

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Molecular Diagnostics

Mats Nilsson

The main aim of our research is to develop improved techniques for molecular analyses. We aim to develop techniques that enable determination of quantities and location of specific nucleic acids in situ; amplified single-molecule detection in solution; and sequence composition of DNA samples.

The basic molecular devices that are used are the padlock- and selector probes, that are both acting through a strictly target dependent ligase-mediated DNA circularization reaction. These reagents have a specificity matching that of PCR, but can unlike PCR be deployed in highly multiplex analyses.

An important objective is to apply these techniques in collaborative projects to solve fundamental research questions and to serve unmet clinical diagnostic needs. In a multidisciplinary approach, dedicated micro and nano devices employing the molecular detection techniques will be developed, to enable rapid, sensitive and cost-effective point-of-care diagnostics.

Mats Nilsson is currently visiting professor at IGP and has since the second half of 2012 his main laboratory at Science for Life Laboratory in Stockholm, being professor at the Department of Biochemistry and Biophysics, Stockholm University (http://www.su.se/profiles/matsn-1.191373). His current group at IGP is mainly engaged in two projects, but he is also engaged in numerous collaborations with other groups at IGP.

Amplified single-molecule detection and biosensors

David Herthnek, Camilla Russell, Malte Kühnemund

An ideal diagnostic analysis device should be able to detect specific biomarkers with single-molecule sensitivity, exquisite specificity, a wide linear quantitative range, high quantitative precision, in a multiplex format, cost effectively and user-friendly. Depending on the purpose, different requirements will apply for such devices, e.g. cheap and simple devices for use in the field in developing countries, simple devices for consumer self diagnostics, rapid and accurate point-of-care devices, and high-throughput - high performance central laboratory devices.

Examples of applications that require extreme sensitivity include the detection of biomarkers leaking from an affected organ into the circulation for early diagnosis of disease, and infectious diagnostics where a single pathogen may be sufficient to cause disease. Moreover, both these applications typically require parallel analyses of large sets of biomarkers.

Sensitive biomolecular analysis requires a highly selective identification reaction coupled to signal amplification that does not introduce background signal noise. Present biosensor and diagnostic devices are limited in one or more of the desired analytic properties. For protein biomarkers, multiplexing and sensitivity are typically limited, while for nucleic acid biomarkers, sensitivity has to be sacrificed to gain multiplexing and the analysis devices are expensive and not very user friendly. We now aim to develop diagnostics concepts by deploying our molecular tools in biosensor devices by utilizing nano- and micro engineering. To that end, we are exploring a simple and sensitive electric read-out within the Berzelii Technology Center for Neurodiagnostics, where we published a proof-of-concept paper recently (see list of publications).

Targeted multiplex genome analysis

Elin Falk-Sörqvist, Lotte Moens, Lucy Mathot

We have developed the selector probe technique, for targeted re-sequencing applications. Our aim is to enable high-performance selective target enrichment as a sample preparation step for next-generation sequencing instruments.

By focusing the sequencing power to the genes and chromosomal regions that are most likely to be relevant for a particular disease, a lot more DNA samples can be sequenced for a certain amount of research funding. In addition, the quality of the sequence can be improved since greater sequencing depth can be afforded, and the data analysis is greatly facilitated compared to sequencing whole genomes. Our main focus is to develop the technology for applications in clinical diagnostics.

Group members during 2015

Mats Nilsson, professor, group leader

Group in Uppsala
Elin Falk Sörqvist, bioinformatician
David Herthnek, post doc
Malte Kühnemund, PhD student
Camilla Russel, PhD student

Group in Stockholm
Annika Ahlford, post doc
Pavankumar Asalapuram, post doc
Anna Engström, post doc
Thomas Hauling, post doc
Ivan Hernandez, PhD student
Tomasz Kryzkowski, PhD student
Anja Mezger, PhD student
Pavankumar Ramachand, post doc
Jessica Svedlund, postdoc

Dissertations during 2015

Camilla Russell, Development of Electrical Readouts for Amplified Single Molecule Detection. May 22, 2015.

Funding during 2015

VINNOVA/VR, Berzelii centre, 280 kSEK

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Molecular Proteomics

Ola Söderberg

My ongoing research and future plans includes both methods development and application of these methods to solve biological and medical problems. These activities are highly interdependent: the need to answer new types of questions is the motivation for methods development, and the availability of novel methods provides opportunities to pursue new scientific challenges.

Although all information about both RNA and proteins is encoded in the DNA, the functional components of a cell are mainly proteins. At any given time point the proteome of each individual cell reflects both genetic and epigenetic information. However, the activity status of proteins is not encoded in the genome. Instead this is regulated by protein-protein interactions and post-translational modifications (PTMs), often as a result of external stimuli mediated by cell-to-cell contacts and binding of secreted proteins.

To deduce the influences of the cellular microenvironment analyses need to be performed of proteins, protein interactions and PTMs at a single cell level *in situ*, thus retaining information of the tissue architecture and positions of all individual cells within this. Targeted analysis utilizing affinity reagents, e.g. antibodies, has been used for decades in both research and for diagnostic purposes. To increase selectivity of affinity reagent based methods, multiple recognition events can be applied to overcome the problem with cross-reactivity, i.e. antibodies that bind to unintended targets. Detection of low abundant molecules requires either sensitive read-out instruments or powerful signal amplification.

Proximity ligation assay (PLA) combines multiple recognitions of affinity reagents with potent signal amplification, utilizing methods for DNA analysis to generate a signal that will be a surrogate marker of the targeted protein, PPI or PTM. The method is based on pairs of proximity-probes (i.e. antibodies conjugated to strands of DNA) to detect the proteins of interest. Only upon proximal binding of these probes can an amplifiable DNA molecule be generated by ligation, which enhance the selectivity of the method even further.

Since the development of *in situ* PLA (Söderberg *et al*., Nat Methods, 2006) most of our efforts has been related to the use of *in situ* PLA and to further improve the method.

Tumor analysis

Linda Arngården, Doroteya Raykova, Johan Heldin, Karin Grannas, Gaëlle Cane

A tumor does not consist of a homogenous population of cancer cells. Therefore, to understand cancer, the tumor microenvironment and the interplay between the different cell types present in the tumor has to be taken into account, and how this interplay regulates the growth and survival of the cancer cells.

The aim with this project is to use *in situ* PLA for simultaneous analysis of the activity status of multiple signal pathways at a single cell level. This will provide information on what pathways are active in cancer cells, and to what extent this varies depending on positioning within the tumor, and in addition it will reveal how the cancer cells affect the surrounding non-malignant cells in the tumor microenvironment. This knowledge will enable better diagnostics, improved prediction on response to therapy and possibly also act as an incitement to develop novel drugs that can modify the microenvironment to reduce cancer growth and ability to metastasize.

Within the project we are developing assays to visualize activity status of pathways that are deregulated in colorectal cancers, such as WNT and EGFR pathways. The assays will be used investigate if analysis of signaling pathway activity in tumor tissue sections will provide

better diagnostics and predictive power than conventional analysis. These assays will also be used for high-content drug screening in primary cell cultures of colorectal cancer samples.

Method development

Karin Grannas, Linda Arngården, Axel Klaesson

Although *in situ* PLA provides the mean to analyze protein interactions and PTMs, further improvements are required to increase the dynamic range, provide ability for multiplex analysis and for visualization of interactions between different types of biomolecules, e.g. proteins and nucleic acids. We are working on increasing the performance on *in situ* PLA, by increasing the efficiency, dynamic range and possibilities to perform parallel analysis. In addition to these PLA based developments, we are developing completely new methods for analysis of protein interactions that will be more robust and inexpensive to facilitate automation and development of point-of-care devices for *in vitro* diagnostics. The recently developed method, proximity dependent initiation of hybridization chain reactions (proxHCR), provides a method that does not require any enzymatic reactions (Koos *et al*., Nat Commun, 2015).

Group members during 2015

Ola Söderberg, senior lecturer, group leader
Linda Arngården, PhD student
Gaëlle Cane, researcher
Elin Ekberg, adm. assistant
Karin Grannas, PhD student
Johan Heldin, post doc
Johanna Herö, research engineer
Axel Klaesson, PhD student
Doroteya Raykova, post doc
Erik Ullerås, project coordinator
Pan Zhou, degree project student

Dissertations during 2015

Karin Grannas, Improvements and Applications of in situ Proximity Ligation Assays. May 29, 2015.

Funding during 2015

EU-FP7 (PRIMES), 2 200 kSEK
EU-IMI (Predect), 1 300 kSEk
Swedish Research Council, 700 kSEK

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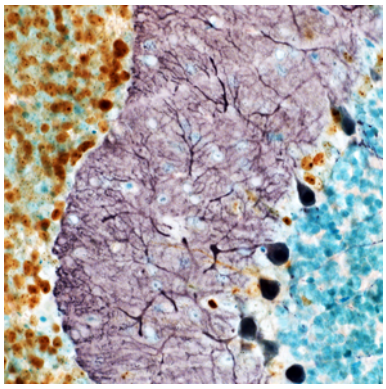
Neuro-oncology



The IGP neuro-oncology program comprises six research labs that employ complementary approaches to study cancers of the nervous system. We focus primarily on two forms of brain tumours, glioblastoma and medulloblastoma. Glioblastoma - which mainly affects adults - is the most frequent form of brain cancer. Currently, the prognosis for glioblastoma patients is very poor and efficient therapies remain to be discovered. Medulloblastoma is the most common primary malignant brain tumour in children. Despite a better prognosis than for glioblastoma many children cannot be cured. In addition, those that survive often suffer from life-long side effects of the aggressive and unspecific standard treatment.

Addressing a major health problem, our labs seek to answer fundamental questions about brain cancer, and to develop new strategies for diagnosis and therapy. For this, we use a broad range of tools, ranging from bio-banks, patient-derived cell models, clinically relevant animal models, and computational modelling. Working with a broad and international network of collaborators, our long-term goal is to introduce new treatments that improve the outcome for patients.

Neural Stem Cells and Brain Tumors

Karin Forsberg Nilsson

The overall goal of our research is an improved treatment of malignant brain tumors, in particular glioblastoma and medulloblastoma. In our projects we incorporate experience of neural stem cells with glioma biology, leveraging the close relationship between these two fields. We also investigate the neuro-inflammatory responses to brain tumors and traumatic brain injury.
Specific goals are:

1. To target the invasive niche of brain tumors with novel experimental therapies (KFN).
2. To establish reliable *in vitro* tumor models and employ these to explore novel regulators of tumor formation (KFN).

Some projects in the group focus on the role of mast cells in brain tumors (EC).

Extracellular matrix interactions of importance for brain tumor formation and neural development

Soumi Kundu, Anqi Xiong, Grzegorz Wicher, Annika Hermansson, Argyris Spyrou, Lulu Rama Haseeb, Misbah Riaz and Andreas Liontos

The focus of this project is the “brain tumor niche” that allows tumor cells to detach from the original site, remodel the extracellular matrix (ECM) and migrate to seed new tumors that ultimately leads to death of the patient. Based on our increased understanding of the biochemical and molecular determinants of brain tumor invasion, new drug targets in the glioma microenvironment could be identified. Heparan sulfate (HS) proteoglycans are main components of the ECM where they interact with a large number of physiologically important

macromolecules, thereby influencing biological processes. HS modulate growth factor activities, and we have shown a vital role for HS in formation of the neural lineage (Forsberg et al., 2012). The major enzymatic activity degrading HS is heparanse. In this project we address HS proteoglycan biosynthesis and degradation in clinical brain tumor samples, human glioma and medulloblastoma cell culture as well as mouse and human models of glioma and medulloblastoma.

Human glioma cell cultures as a new experimental platform

Grzegorz Wicher, Annika Hermansson, Argyris Spyrou, Lulu Rama Haseeb

Basic cancer research, including preclinical tumor models and testing of candidate drugs needs optimized in vitro models that better reflect the patient's disease. There are major challenges in generating model systems at the scale necessary to demonstrate patient tumor heterogeneity. The availability of "tumor stem cell" culture techniques has opened the possibility to create well-characterized human tumor cell cultures. However, to establish these experimental tools requires simultaneous access to the technical know-how of culturing and analyzing cancer cells, and a systematic biobanking pipeline of patient tissue combined with clinical data acquisition. All these parameters are now in place at the Rudbeck Laboratory through a collaborative effort between K. Forsberg Nilsson, L. Uhrbom, B. Westermark, and S. Nelander, clinical collaborators G. Hesselager and I. Alafuzoff, Uppsala University Hospital and the U-CAN project (www.u-can.uu.se).

Investigating regulators for brain tumors and neural stem cells

Anqi Xiong and Karl Holmberg Olausson

We previously reported that malignant brain tumors and neural stem cells share a common transcriptional signature (Demoulin et al, 2006) and selected the pseudokinase nuclear receptor binding protein 2 (NRBP2), for further study because of the high level of regulation (Larsson et al, 2008). Pseudokinases have high sequence similarity to mechanistically validated enzymes, but are devoid of the catalytic activity (NRBP2 lacks 7 out of 15 residues of the kinase domain) and are now increasingly viewed as components of signaling pathways. We are now working to identify the function of NRBP2 and its role in brain tumor development.

Dogs provide valuable spontaneous models for complex human diseases and certain dog breeds exhibit a considerably elevated risk of developing glioma, We have identified a genomic region associated strongly with glioma in dogs (Truvé et al, manuscript) and will explore candidate genes, expressed differentially in glioma and the healthy brain, for their roles in tumor development.

The role of IL-33 in development, brain injury and brain tumors

Grzegorz Wicher and Andreas Liontos

IL-33 has important functions in inflammatory and autoimmune diseases (Enoksson et al, 2013). Little is known, however, about IL-33 in brain development, injury and brain tumors. Our data suggest that IL-33 expression is under tight regulation in the normal brain but can be triggered by injury. Its detection during the first three weeks of postnatal life coincides with important parts of the CNS developmental programs, and opens the possibility of IL-33 involvement in normal developmental processes (Wicher et al, 2013). De novo expression of IL-33 after injury suggests involvement of this alarmin in the neuro inflammatory response. A high level of expression in glioma samples implies a role in tumor development and progression.

The role of mast cells in gliomagenesis

Elena Chugunova, Sanaz Attarha, Ananya Roy, Anna Sjösten

Human cancers maintain a complex inflammatory program triggering rapid recruitment of inflammatory cells, including mast cells (MCs), to the tumor site. MCs are crucial players in various inflammatory conditions, including cancer. The potential contribution of MCs in glioma has not been addressed previously.

Just recently we have expanded our understanding of the role of inflammation in gliomas by showing, for the first time, that MCs infiltrate mouse and human glioma, and that the extent of MC infiltration, both in mouse and human gliomas, shows a strong positive correlation with the malignancy grade of the tumor.

Considering novel data it becomes increasingly important to thoroughly elucidate new trends in interactions between MCs and glioma. i) The revealing of pro- or antitumorigenic role of MCs upon glioma development and presumably opposing MC functions depending on glioma grade. ii) The determination of conditions at which glioma cells cause the potential functional switch in MCs and iii) To what extent the parallels can be made between the well-defined mouse model and poorly understood human condition.

Mast cell contribution to brain metastasis

Elena Chugunova, Ananya Roy, Sanaz Attarha, Ida Gustavsson

Brain metastases are becoming an important problem because of the progressive neurological disability and the lack of effective treatment due to the unique structure of the blood-brain-barrier (BBB). Recent studies in this field point towards a link between the immune system and metastases pathogenesis but many aspects still need to be investigated. In order to clarify the role of MCs and other immune cells in brain metastasis we aim to understand the mechanisms underlying the MC-brain metastatic cell interactions and identify key factors regulating these interactions.

Our preliminary data, for the first time, demonstrated the abundant accumulation of MCs in human brain metastases originated from different primary tumors (lung, prostate, kidney, ovarian and rectum). We expect MCs to contribute to the expansion of angiogenesis within brain metastases with specific addressing the role for MC proteases in this process. We plan to investigate MC-brain metastases cell interaction (*in vitro* studies), early stages of brain metastasis development (*in vivo* studies), as well as gather clinical data by exploring patient brain metastases samples and corresponding primary tumors. Our final goal is to reveal the correlation in these studies and support it with mechanistic findings.

Group members during 2015

Karin Forsberg Nilsson, professor, group leader
Annika Hermansson, research engineer
Karl Holmberg Olausson, post doc
Suomi Kundu, researcher
Andreas Liontos, degree project student
Lulu Rahma Adil Haseeb, PhD student
Misbah Riaz, degree project student
Argyris Spyrou, PhD student
Grzegorz Wicher, researcher
Anqi Xiong, PhD student

Group member establishing independent research
Elena Chugunova, researcher
Sanaz Attarha, post doc
Ida Gustavsson, student
Ananya Roy, researcher
Anna Sjösten, PhD student

Dissertations during 2015

Anqi Xiong, Novel Regulators of Brain Tumor Development: – From neural stem cell differentiation to in vivo models. December 15, 2015.

Funding during 2015

Karin Forsberg Nilsson
Swedish Research Council, 700 kSEK
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Swedish Childhood Cancer Foundation, 400 kSEK
Swedish Childhood Cancer Foundation NBCNS grant 400 kSEK

Elena Chugunova
Swedish Cancer Society, 600 kSEK

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Predictive oncology: systems scale analysis and prospective modelling of cancer

Sven Nelander

I am a cancer systems biologist, focusing on both experimental and computational aspects of new cancer therapies. My research group comprises 15 staff with both experimental and computational backgrounds. Key areas of interest include regulatory networks and drug response in glioblastoma stem cells, fundamental aspects of brain tumour progression, and innovative methodology for precision medicine.

Precise targeting of cellular networks in brain tumor stem cells.

Our lab is developing novel strategies to enable efficient network-based drug development to target cancer stem cells (CSCs). For this, we work with partners to establish a unique Uppsala based biobank of more than 150 patient-derived CSC cultures, from patients with brain cancer (glioblastoma). To enable a systems biological analysis, each cell line is systematically characterised at multiple levels. Unlike traditional biobank studies, our characterisation includes both functional and molecular data, ranging from mutations, to epigenomics to comprehensive knockdown screening information. We then construct computational models that aim to increase our understanding of CSC biology, including:

1. *Which are the mechanisms that drive key phenotypes of the CSCs, like tumour initiation capability?*
2. *Which are the mechanisms that make mediate functional heterogeneity, e.g. differences in drug response between two patients?*
3. *How can we optimally intervene optimally to suppress disease progression or prevent recurrence after surgery?*

An important unique aspect of this study is the integration between a state of the art biobank with computational modelling of extensive data. The effort thus has potential to unravel new therapies, patient prognostics and biomarkers. Our effort is highly inter-disciplinary and involves collaborations with the IGP neurooncology groups, SciLifeLab platforms as well as international partners.

Big data integrative models of cancer

The ongoing efforts worldwide to develop cancer therapies are increasingly dependent on accurate data analytics. My lab develops new methods computational methods and mathematical models that will help researchers to interpret complex cancer data sets. Key challenges that we are addressing are:

1. *How can multiple sets of cancer information be computationally integrated into models that help us understand cancer mechanisms?*
2. *Can we predict strategies to protect non-cancerous tissue from side effects of cancer therapies?*
3. *How do we best design combinatorial interventions against cancer cells?*

Addressing these multi-faceted questions, we combine both unqiue, in-house data sources, as well as multiple layers of public data. A key component of the work is also to make our results available as tools and packages that can be used by cancer researchers. One recent example is Cancerlandscapes.org (NAR 2015).

Group members during 2015

Sven Nelander, associate professor, group leader
Elin Almstedt, PhD student
Olatilewa Awe, guest researcher
Satishkumar Baskaran, PhD student
Ludmila Elfineh, research engineer
Evgenia Gubanova, post doc
Karl Holmberg Olausson, post doc
Patrik Johansson, PhD student
Marianne Kastemar, technician
Soumi Kundu, researcher
Cecilia Krona, researcher
Ingrid Lönnstedt, researcher
Linnéa Schmidt, affiliated researcher
Jenny Lindvall, student
Ismail Hermelo, student

Funding during 2015

Swedish Research Council, 1 125 kSEK
Swedish Cancer Society, 1 000 kSEK
Swedish Childohood Cancer Foundation, 450 kSEK
AstraZeneca, 5 630 kSEK
eSSENCE, 400 kSEK

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Development of Childhood Brain Tumors and Targeting of MYC Proteins

Fredrik Swartling

MYC proteins (like MYC or MYCN) are transcription factors and potent mitogens with essential roles in normal brain development. Misexpression of MYC proteins occurs frequently in medulloblastoma, the most common malignant childhood brain tumor of the hindbrain. *MYC* or *MYCN* amplifications in medulloblastoma are strongly correlated with poor prognosis suggesting MYC proteins are clinically relevant targets for brain tumor therapy. MYC proteins are also amplified or overexpressed in childhood pons glioma (DIPG) of the brain stem and in adult glioma, adult malignant brain tumors of the forebrain.

Our research group is exploring how MYC proteins are stabilized in malignant brain tumors with a focus on identifying cells of tumor origin. We further study critical pathways involved in tumor recurrence and new treatments for MYC/MYCN-driven brain tumors. We have generated clinically relevant models for MYC/MYCN-driven brain tumors and we also study a large number of primary cell lines obtained from childhood brain tumor patients.

In search for the cellular origin of MYCN-driven medulloblastoma

Sara Bolin, Holger Weishaupt and Fredrik Swartling

We recently showed that MYCN could generate tumors from a glutamate transporter (GLT1) promoter in a transgenic inducible model (GTML) of medulloblastoma (Swartling et al. Genes & Dev., 2010). By mapping cellular fate we found that GLT1-positive neural stem cells (NSCs) represent putative cells of brain tumor origin. GTML mice generate aggressive medulloblastoma after about 3-6 months. Before tumor onset we found significantly more proliferating cells in thalamic forebrain cells and of cerebellar Bergmann glia as compared to controls. Currently we study cellular fate using various brain cell-specific promoters to understand how these tumors develop. We are also isolating putative cells of tumor origin using laser-capture microdissection. Detailed bioinformatic analysis of expression profiles of distinct brain cells is performed in order to reveal the cellular origin for these malignancies.

FBW7 regulates MYCN protein stabilization during brain tumor formation

Vasil Savov, Sanna-Maria Hede, Sara Bolin and Fredrik Swartling

Medulloblastoma is divided into four distinct molecular subtypes (WNT, SHH, Group 3 and Group 4). Group 3 and 4 tumors often show amplifications of MYC and MYCN, respectively, and correlate with poor prognosis. MYC proteins are unstable oncoproteins with short half-lifes. We recently found that stabilization of MYCN is essential for brain tumor initiation (Swartling et al. Cancer Cell, 2012). MYCN stability is regulated by the ubiquitin ligase FBW7, which normally targets it for proteasomal degradation. FBW7 is a tumor suppressor gene mutated in various types of cancer including medulloblastoma and we study loss of function of FBW7 in our animal models of medulloblastoma. We have crossed FBW7 knock-out mice to GTML mice and currently study how FBW7 loss alters brain tumor formation.

A new model for childhood brain tumor recurrence

Vasil Savov, Gabriela Rosén, Sara Bolin, Holger Weishaupt and Fredrik Swartling

Tumor recurrence is the main cause of death in children with medulloblastoma. In this project we are studying how MYCN interacts with SOX9, a transcription factor involved in glial fate determination in the brain. Few scattered SOX9-positive cells are found in GTML tumors that are similar to Group 3 or Group 4 human MB. By using a combination of Tet-ON and Tet-OFF inducible systems we managed to target this rare population of SOX9-positive GTML

tumor cells *in vivo* to show how they were capable of initiating tumor recurrence. The relapsed tumors develop at a distant site in the brain, in line with recent patient data. Further, isolated metastases in Group 3/4 patients had consistently higher SOX9 levels as compared to corresponding primary tumors. We also showed how FBW7 is regulating SOX9 stability and increases tumor cell migration and metastasis. By suppressing the mTOR/PI3K/AKT pathway we can obstruct this stabilization. Further characterization of SOX9-positive tumor cells will help us understand the mechanisms behind metastatic medulloblastoma recurrence.

Targeting MYCN through Bromodomains and by using CDK2 inhibitors

Sara Bolin, Holger Weishaupt, Anders Sundström and Fredrik Swartling

We recently showed that MYCN levels and early proliferation of brain tumors could be reduced by specific inhibition of the bromodomain inhibitor JQ1, which targets MYC proteins epigenetically (Bandopadhayay et al. Clin Can Res., 2014). We also found good efficacy controlling MYCN stabilization by using a CDK2 inhibitor called Milciclib. Both drugs induced tumor cell senescence or apoptosis in our brain tumor models and also in primary human brain tumor cells. As compared to either drug alone, when combining the two drugs we further reduced MYCN levels and completely abolished brain tumor growth after long-term treatment in vitro. We are currently evaluating these treatment effects in our models in vivo. Our goal is to understand the underlying mechanisms of this MYCN inhibition and further evaluate the potential of using these promising drugs in the clinic.

Using human hindbrain cells to study medulloblastoma and DIPG development

Matko Čančer, Sonja Hutter, Anna Borgenvik, Geraldine Giraud, Holger Weishaupt and Fredrik Swartling

In this project we are transforming human hindbrain neural stem cells in order to model the different subgroups of medulloblastoma using lentiviruses carrying clinically relevant cancer driver genes for the distinct tumor subgroups. We are also transforming brain stem-specific cells from humans and mice in order to model diffuse-intrinsic pontine glioma (DIPG) development. We will evaluate the relevance of using well-defined human hindbrain stem cells to generate these childhood brain tumors and we will compare them to subtype-specific cells similarly cultured from medulloblastoma or DIPG patients. We hope we will understand what actually drives the initiation of medulloblastoma and DIPGs and if various subgroups match certain hindbrain cell types. Finally, we will use genetic and epigenetic analyses to predict how these cells could be treated or if they would be resistant to targeted therapies.

A forward genetic screen to identify cancer-causing genes in brain tumors

Holger Weishaupt, Matko Čančer, Sonja Hutter, Gabriela Rosén, Sara Bolin and Fredrik Swartling

We use a tumor model to study human glioma development from cell-type specific and retrovirus-driven Platelet-Derived Growth Factor (PDGF)-B overexpression. We further use Piggy Back retrotransposons from where we overexpress MYCN expression to induce childhood brain tumors. Virus or transposon integration into the host genome presents a risk for insertional mutagenesis, which can alter proximate genes, giving a particular tumor cell an advantage over other cells during tumorigenesis. We have used genome sequencing to identify genes that, together with PDGF and MYCN, can contribute to tumor development. We have developed a streamlined analysis pipeline for integration detection, followed by integration site annotation against functional genes and enhancers. We hope this technique will enable us to identify important brain tumor-causing genes. The most promising genes are functionally evaluated in order to understand their role in the tumor initiation process.

Group members during 2015

Fredrik Swartling, researcher, group leader
Sara Bolin, PhD student
Anna Borgenvik, PhD student
Matko Čančer, PhD student
Sanna-Maria Hede, post doc
Lisa Franziska Drews, degree project student
Geraldine Giraud, post doc
Sonja Hutter, post doc
Gabriela Rosen, lab technician
Hanna Sabelström, post doc
Vasil Savov, PhD student
Anders Sundström, research engineer
Holger Weishaupt, post doc

Funding during 2015

ERC, 2 000 kSEK
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Swedish Cancer Society, 500 kSEK
Swedish Childhood Cancer Foundation, 400 kSEK

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A Cell of Origin-Based Strategy to Decipher Glioma Biology

Lene Uhrbom

Glioma is a large and heterogenous group of primary CNS tumors comprising astrocytoma, oligodendroglioma and ependymoma of all malignancy grades (I-IV). Glioma can strike at any age but the majority of patients are adults. Only grade I tumors are benign while grade II-IV tumors are malignant. Glioblastoma is a grade IV glioma and the most common form of all primary malignant brain tumors with dismal prognosis and essentially no cure. In my group we study many types of malignant glioma with a particular interest in glioblastoma. Recent large-scale efforts to uncover the genetic and epigenetic landscape of glioma has led to a comprehensive molecular characterization of the important oncogenic pathways and reveal the vast inter- and intratumor heterogeneity of these tumors. This has produced a molecular subtype classification where tumors with common genetic and epigenetic signatures are grouped. Although informative about the biology of malignant glioma this classification has not yet provided any breakthrough in the clinic.

The cell of origin for glioma, including glioblastoma, remains unknown. It is generally presumed to be a neural stem cell or glial progenitor cell but this has not been formally proven. For a complete understanding of glioma biology we believe that it is essential to understand from where a tumor originates and how that will affect the phenotype of the cancer cells. My research is focused on understanding how the cell of origin in combination with various glioma-relevant genetic alterations affects tumor development, progression and response to treatment. By integrating in vivo and in vitro studies and using a cross-species bioinformatics approach the goal is to uncover genes, mechanisms, pathways and targets to which directed therapies can be developed.

Our studies are mainly carried out using life-like glioma mouse models and a new and continuously growing biobank of human glioma cell cultures (HGCC) established from patient surgical samples. The HGCC biobank is established and maintained in collaboration with Karin Forsberg-Nilsson, Sven Nelander and Bengt Westermark. In all, our mouse and human glioma models provide a unique and relevant platform for our basic and pre-clinical glioma research.

Projects

- **Establishment of the HGCC biobank of cultured glioblastoma cells**.
  Yuan Xie, Prathyusha Maturi and E-Jean Tan, in collaboration with Karin Forsberg-Nilsson, Bengt Westermark and Sven Nelander
- **Cell of origin for glioblastoma as a basis for stratification, target identification and drug screening**.
  Yuan Xie, in collaboration with Yiwen Jiang, Voichita Marinescu, Sven Nelander, Rolf Larsson, Mårten Fryknäs, Malin Jarvius and Caroline Haglund
- **The interplay between cell of origin, oncogenic activation and developmental age in glioma development**.
  Smitha Sreedharan, Prathyusha Maturi, Yuan Xie, Anders Sundström
- **Role of LGR5 in glioma stem cells**.
  Yuan Xie, E-Jean Tan and Anders Sundström
- **Investigations of human glioblastoma cell cultures of the mesenchymal subtype**.
  E-Jean Tan, Prathyusha Maturi and Yuan Xie

Group members 2015

Lene Uhrbom, senior lecturer, group leader
Ann-Charlotte Hellström, research technician
Naga Prathyusha Maturi, research assistant
Smitha Sreedharan, post doc
Anders Sundström, research engineer
E-Jean Tan, post doc
Yuan Xie, PhD student

Funding during 2015

Swedish Resarch Council, 700 kSEK
Swedish Cancer Society, project grant, 800 kSEK
Swedish Cancer Society, Senior Investigator Award, 1 102 kSEK
Swedish Childhood Cancer Foundation, 400 kSEK

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Human Malignant Glioma – from Oncogenic Mechanisms to Treatment

Bengt Westermark

Our research is focused on glioblastoma, the most common form of malignant brain tumors in adults. Our main goal is to understand the molecular mechanisms of glioblastoma development. This knowledge may increase the possibilities of developing novel treatment modalities.

Human glioblastoma cell lines are established from fresh tumor surgical specimens taken in connection with brain tumor surgery. The aim here is to identify novel lead substances that inhibit tumor cell growth.

CGGBP1 in cell cycle checkpoint regulation and telomere protection

Umashankar Singh, Bengt Westermark

Using a genetic screen in mice, we have identified a number of glioblastoma candidate genes. One of these, NFIX, was used as a bait to find binding partners in a yeast-two-hybrid screen. One of the binding partners, the transcriptional regulator CGGBP1 is involved in DNA damage response, localizes to midbodies, regulates abscission and prevents tetraploidy. A novel role of CGGPB1 as a protector of teleomeres was studied in human diploid fibroblasts. Expression of a mutated form of CGGBP1, in which an ATR phosporylation site (Serine-164) has been mutated, leads to telomere shortening, DNA damage response at telomeres, telomere fusions resulting in chromatin bridges between dividing cells, and cell cycle arrest. The finer mechanistic details of CGGBP1 as a protector of telomeres are being analyzed. Further, we have found by chromatin immunoprecipitation sequencing that CGGBP1 binds to repetitive DNA sequences of the LINE1 and Alu families and regulates Alu expression.

Search for candidate drugs for the treatment of malignant glioma

Anna Segerman, Bo Segerman, Mia Niklasson, Tobias Bergström, Erika Dalmo, Bengt Westermark

We aim to identify novel targets and lead substances with the ultimate goal to improve glioblastoma therapy. While taking tumor heterogeneity into account, we will characterize the subgroup of tumor cells with relapse potential (glioma initiating cells, GICs).

Glioma cell lines are continuously established from fresh biopsies and characterized with regard to genotype (structural alterations in known oncogenes and suppressor genes), phenotype (e.g. expression of stem cells and differentiation markers and tumorigenicity in immunocompromized mice) and treatment response using the standard glioma regimen (radiation and temozolomide). Selected cell lines and clonal derivatives are subjected to transcriptome and proteome analysis to define biomarker signatures.

Using growth inhibition as endpoint, we analyze the response of individual glioma cell lines to BMP4. Using CRISP-Cas9 knock out technology, we aim to define BMP4 signaling in growth inhibition. Further, chemical libraries of a total of >20,000 compounds will be used for high throughput screening. After substantial in vitro testing, the efficacy of the identified substances and combinations will be analyzed in vivo (orthotopic xenotransplantation in mice).

The role of Sox21 as a suppressor gene during glioma progression

Maria Ferletta, Erika Dalmo, Bengt Westermark

The transcription factor Sox2 is required for maintaining the pluripotency of embryonal stem cells. Sox2 is expressed in neuronal stem cells and down regulation of Sox2 is accompanied

by neuronal differentiation. We have shown that both Sox2 and Sox21 are expressed in adult and pediatric brain tumors and that the expressions of the transcription factors are correlated. Our *in vitro* studies indicate that Sox21 can down regulate Sox2 in glioma cells and the *in vivo* studies show that an up regulation of Sox21 decreases the tumor growth significantly as well as prolong the survival extensively. Sox21 appears to decrease the stem-like cell properties of the tumor cells and induce abnormal differentiation and apoptosis as well as reduce cell proliferation in glioma cell *in vivo*. Further, tumor cells with increased expression of Sox21 demonstrated an improved formation of Sox2:Sox21 complexes. Our studies indicate that Sox21 function as a tumor suppressor during gliomagenesis mediated by a shift in the complex formation of Sox2:Sox21. These results imply that the Sox2/Sox21 axis could be a potential therapeutic component.

So far very little is known about which signaling pathways Sox21 take part in, so to investigate that we have performed cDNA arrays to identify signaling pathways and components important for mediating the suppressor effect of Sox21 in glioma cells. We are at the moment focusing on the TGF-β/BMP4 signaling pathways and the JAK/STAT-signaling pathway. In addition we will study if theses signaling pathways or if the Sox2/Sox21 axis can be inhibited by low molecular weight inhibitors to prevent brain tumor progression.

Molecular studies of growth and carcinogenesis in the thyroid gland

Nils-Erik Heldin

Undifferentiated (anaplastic) tumours are highly malignant, rapidly growing and invasive, and constitute a major clinical problem. This project focuses on anaplastic thyroid carcinoma (ATC) and our aim is to elucidate the genetic events involved in generating the tumour.

Our laboratory has established several cell lines from human anaplastic thyroid cancer biopsies. Analyses of their karyotypes showed an abundance of double minute chromosomes (DMs) in two of the cell lines. DMs are known to harbour amplified gene sequences. With this in mind, we are currently using “next generation” sequencing technology to identify the amplified sequences.

Group members during 2015

Bengt Westermark, professor, group leader
Tobias Bergström, post doc
Erika Dalmo, research engineer
Nils-Erik Heldin, associate professor
Mia Niklasson, researcher
Anna Segerman, researcher
Bo Segerman, associate professor
Jacob Wall, student
Ann Westermark, teaching assistant

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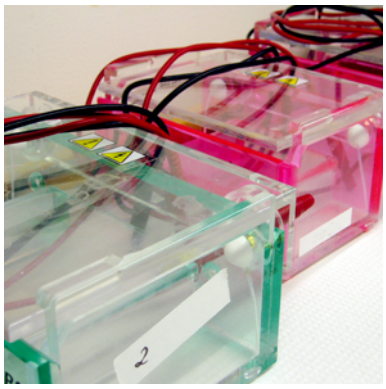
Vascular Biology



The formation of new blood vessels, angiogenesis, is an important and strictly controlled process that under normal circumstances takes place during embryonic development, in wound healing and in the female menstruation cycle. However, in several diseases, for instance cancer, there is an exaggerated angiogenesis that leads to a disorganized and dysfunctional vasculature that may propagate the disease.

In the research programme *Vascular Biology* we study how angiogenesis is regulated, both during embryo development, in adults and in diseases, mainly cancer. We are particularly interested in how growth factors and other regulating proteins stimulate or inhibit angiogenesis during development, and how vessel permeability to molecules and cells is regulated in the CNS and in peripheral organs. We also study the mechanisms underlying the formation of functional lymphatic vessels and the development of fibrosis.

Developmental Genetics

Christer Betsholtz

Our group studies cellular and molecular mechanisms of angiogenesis, vascular permeability and other vascular functions (vessel tone, molecule transport, cell transmigration across the vessel wall), in embryonic development, adult homeostasis and disease.

A particular focus is placed on the microvascular pericyte. Pericytes are obligatory components of all blood capillaries, yet their functions in health and disease are still poorly understood. Our on-going research addresses pericyte functions in different situations in organs using *in vivo* and *in vitro* techniques.

Other areas of focus concern the mechanisms of angiogenic sprouting, and the specific role of G-protein coupled receptors in this process as well as in other microvascular functions. A large project relates to the blood-brain barrier (BBB), a complex and specific feature of the neurovascular unit, and the role of pericytes in this structure.

Some of our questions go beyond vascular biology. In a broad sense we address the roles of platelet-derived growth factors (PDGFs) and other growth factors and their intracellular signal transducers during embryonic and postnatal development, as well as in pathological processes in the adult organism, such as cancer and brain calcification and neurodegeneration.

Mechanisms of angiogenesis and vascular permeability: the role of G-protein coupled receptors.

Konstantin Gaengel, Colin Niaudet, Barbara Lavina-Siemsen, Marco Castro

We previously identified a core set of 58 gene transcripts expressed specifically (and quite universally) in endothelial cells. This set of genes included some 20 well-established endothelial markers, many of which are known to play critical roles in vasculogenesis and angiogenesis.

Interestingly, however, approximately half of the 58 gene transcripts had not been previously implicated in vascular biology. Many of them are highly interesting as candidate

novel regulators of angiogenesis since they 1) are highly endothelial-specific in their expression, and 2) encode proteins predicted to play a role in cell signaling, such as GPCRs. In our current research program, we are investigating cellular and molecular mechanisms involved in angiogenesis, with focus on new regulators and regulatory processes involved in vascular morphogenesis, stabilization and barrier formation.

Analyses of PDGF signaling during organ development

Johanna Andrae, Leonor Gouveia

The overall aim for this project is to analyze and describe developmental processes where members of the platelet-derived growth factor (PDGF) family play important roles. We focus on processes that are dependent on proper signaling through the tyrosine-kinase receptor PDGFRα. Generally viewed PDGFRα is expressed by mesenchymal and glial cells, whereas adjacent epithelial, muscle or neuronal cells express the ligands PDGF-A and/or PDGF-C. This is true for example in brain, lung, intestine, palate and hair follicles.

Our main goal is a detailed understanding of how correct PDGF signaling contributes to developmental processes in lung and the central nervous system. What are the characteristics of the cells that express PDGFRα? Where are they located in relation to the ligand expressing cells? What happens to those cells in the absence of PDGF, or if they are over-stimulated? It is important to know how different cells contribute to a specific tissue organization.

All cell types use specific molecular signals to communicate with each other, and knowing the normal signaling pathways may be crucial for understanding a pathological behaviour.

Pericyte biology and markers

Bongnam Jung, Michael Vanlandewijck

Pericytes are essential for development and stabilization of the vascular networks. These cells also regulate capillary blood flow, and are a component of the neurovascular unit that controls blood-brain permeability. In addition, immune, phagocytic and contractile functions are assigned to pericytes. Genetic mutation and cell-based studies have demonstrated pericyte engagement in physiological functions and in diseases, including vascular/ organ development, wound healing, scarring, fibrosis and tissue remodeling. For example, PDGF-B or PDGFRb- deficient mice die perinatally exhibiting vascular dysfunction due to a lack of pericyte investment around blood vessels, suggesting the critical role of PDGFB/R signaling in vascular maturation.

Although the biological significance of pericytes is appreciated, a lack of pericyte-specific markers have hampered in-depth study on their origin, presence and function during physiological and pathological processes. To date, existing pericyte markers, such as PDGFRβ, NG2, desmin and CD13, cannot distinguish pericytes from vascular smooth muscle cells (vSMCs) or other mesenchymal cells. The expression patterns of these markers also vary between species, developmental stages and tissues. Therefore, 1) pioneering a reliable, pericyte-specific marker and 2) characterizing known marker expression in a timely- and organ- specific manner are necessary for proper analysis of pericyte biology in health and disease.

We take advantage of the double fluorescent transgenic mouse model, PDGFRβ-EGFP/NG2-dsRed, to study pericyte expression in embryonic and adult mouse tissues by immunofluorescence staining and imaging. Further, we use these mice to FACS pericytes for deep sequencing-based transcriptional profiling to investigate not only novel and specific pericyte markers but also transcriptional differences in pericytes from various organs. Utilizing the PDGF knock out mice crossed to the NG2-dsRed mouse, we hope to address the

precise mechanism of PDGFs in regulation of pericyte function, and differential behavior of pericytes throughout development and in adulthood.

Zebrafish models

Lwaki Ebarasi

We use the zebrafish as a model organism for the study of angiogenesis, pericyte and mesangial cell biology, glomerular development and function in the context of the developing zebrafish embryo. We exploit the experimental advantages of rapid development, transparency, *ex utero* development, and a rapidly expanding arsenal of genetic tools to explore the cellular interplay and molecular regulators of these cells and processes.

Organogenesis and patterning are complete in the first two days of the developing zebrafish embryo's life. Endothelial, podocyte, erythrocyte, tubular, and astrocyte cell-specific reporter lines are some of the tools we apply in our research. The mechanisms, cell types and molecular regulation of angiogenesis and glomerular development and function in the zebrafish are the same ones at play in the higher vertebrates. We apply both forward and reverse genetic approaches to elucidate the molecular mechanisms important to endothelial cell and glomerular development, homeostasis, and function as well as high-resolution live imaging to study cellular behavior and interactions.

The Angiopoietin/Tek system in fibrosis

Marie Jeansson

Angiopoietins are proteins that bind the tyrosin kinase receptor Tek (also called Tie2), expressed on the endothelium of blood vessels. Angiopoietin-1 is an agonist and results in stabilization and quiescence of the vessel whereas Angiopoietin-2 is an antagonist and inhibits the protective Angiopoietin-1/Tek signaling.

Several clinical conditions, including cardiovascular disease, malaria, and sepsis, increase the serum level of Angiopoietin-2, and the increased ratio between Angpiopoietin-2/Angiopoietin-1 has been shown to predict adverse outcomes. One of our objectives is to define the role of the Angiopoietin/Tek system in fibrotic diseases. To do this we are utilizing inducible conditional knockout mice for different components of the angiopoietin system in different models of fibrosis. We are also using RNASeq to identify new targets in the angiopoietin system that may affect fibrosis.

Group members during 2015

Christer Betsholtz, professor, group leader
Alberto Alvarez, scholarship fellow
Maarja Andaloussi Mäe, researcher
Johanna Andrae, researcher
Marco Castro, PhD student
Jana Chmielniakova, technician
Lwaki Ebarasi, post doc
Maria Leonor Segurado Gouveia, PhD student
Rajesh Gupta, researcher
Konstantin Gängel, research fellow
Liqun He, researcher
Jennifer Hofmann, post doc
Bongnam Jung, post doc
Barbara Lavina Siemsen, researcher

Helene Leksell, biomedical analyst
Khayrun Nahar, PhD student
Colin Niaudet, researcher
Cecilia Olsson, technician
Pia Peterson, technician
Milena Petkova, degree project student
Sahar Al Sayegh, degree project student
Michael Vanlandewijck, post doc

Group member establishing independent research
Marie Jeansson, researcher
Krishnapriya Loganathan, PhD student
Ebtisam Salem Said, degree project student
Sedigheh Naseri, degree project student
Emily Winterrowd, degree project student

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Christer Betsholtz
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Marie Jeansson
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Regulation of Blood Vessel Formation

Lena Claesson-Welsh

Vascular endothelial growth factors (VEGF) are essential regulators of blood vessel formation, angiogenesis, and survival of existing blood vessels. VEGF was originally denoted VPF, vascular permeability factor (VPF), reflecting the essential role of VEGF in regulation of molecular flow across the vascular wall (denoted vascular leakage). VEGF exerts its effect by binding and inducing dimerization of receptor tyrosine kinases, VEGFR1 and VEGFR2, on endothelial and lymphendothelial cells. VEGFR2 is the most important receptor for VEGF; activation of VEGFR2 by VEGF is essential for development of the vasculature during embryogenesis and for regulation of angiogenesis in physiological and pathological processes.

We employ *in vivo* models to study VEGF signal transduction in healthy organs, and in disease such as cancer in tumors, retinopathy and myocardial infarction. Our particular interest is to identify signal transduction pathways regulating essential biological effects of VEGF such as endothelial survival, proliferation and vascular leakage with the ultimate goal to specifically inhibit such pathways by small molecular weight inhibitors. We are moreover interested in how VEGF signaling is influenced by the coreceptor neuropilin1, and by other pathways regulating the stability of endothelial junctions, e.g. via the actin cytoskeleton. We furthermore study the biology of the heparin-binding plasma protein histidine-rich glycoprotein (HRG), which acts on inflammatory cells and indirectly, on blood vessels. Treatment with HRG normalizes tumor vessels, and decreases metastatic dissemination. One important goal of our research is to exploit our findings for therapeutic applications.

Regulation of inflammation and angiogenesis by histidine-rich glycoprotein (HRG)

Hiroshi Kaito, Frank Roche

The heparin-binding plasma protein HRG was originally identified as a regulator of tumor angiogenesis. We have shown in a number of models that expression of HRG in tumors results in reduced primary tumor growth and reduced metastatic spread. These effects of HRG depends on polarization of macrophages from an M2 to an M1 phenotype, accompanied by reduced production of angiogenic growth factors and promotion of an anti-tumor immune response. Iodinated HRG binds specifically to mononuclear phagocytes but also to integrins expressed on endothelial cells (Roche et al., 2015). Current aims include to identify the HRG-binding molecule, the HRG receptor, on mononuclear phagocytes, and to explore the potential therapeutic benefit of HRG in combinatorial cancer immunotherapy.

Regulation of angiogenesis and vascular leakage

Daisuke Fukuhara, Emma Gordon, Marie Hedlund, Naoki Honkura, Xiujuan Li, Eric Morin, Elisabet Ohlin Sjöström, Narendra Padhan, Frank Roche, Miguel Sainz Jaspeado, Ross Smith, Chiara Testini, Charlotte Wikner

Dysregulation of VEGF and its receptor VEGFR2 in tumors leads to exaggerated formation of leaky and dysfunctional vessels, which in turn promotes tumor invasiveness and metastatic spread. We have identified the *in vivo* signal transduction pathway regulating vascular leakage in response to VEGF. The pathway is initiated by phosphorylation of tyrosine 949 in VEGFR2, which allows binding of the Src Homology 2 (SH2) domain-containing adaptor molecule TSAd (T cell specific adaptor) that in turn couples to the cytoplasmic kinase c-Src. c-Src becomes translocated to endothelial cell junctions where it phosphorylates the important

adherens junction component vascular endothelial cadherin. Gene targeting to eliminate Y949 or TSAd specifically in endothelial cells results in a block in VEGF-induced vascular leakage and thereby reduced edema and suppressed metastatic spread in a number of mouse tumor models (melanoma, glioblastoma, insulinoma) and myocardial infarction (in collaboration with Prof. Jan Borén, Sahlgrenska Academy). Drug screening is ongoing to identify a drug that blocks the Y949-TSAd-c-Src pathway. We moreover study the dynamics of the transient opening of endothelial junctions using live microscopy. In a parallel project, we examine the biology of other VEGFR2 phosphotyrosine sites such as Y1212.

We furthermore address the role of VEGF co-receptors (heparan sulfate and neuropilin) in presentation of VEGF to VEGFR2, their ability to regulate VEGFR2 internalization and the subsequent biological response.

Group members during 2015

Lena Claesson-Welsh, professor, group leader
Jeremy Adler, research engineer
Katie Bentley, researcher
Daisuke Fukuhara, post doc
Emma Gordon, post doc
Marie Hedlund, research engineer
Naoki Honkura, post doc
Hiroshi Kaito, post doc
Xiujuan Li, researcher
Arindam Majumdar, guest researcher
Eric Morin, PhD student
Elisabet Ohlin Sjöström, researcher
Narendra Padhan, post doc
Tor Persson Skare, PhD student
Frank Roche, post doc
Miguel Sainz Jaspeado, post doc
Ross Smith, PhD student
Chiara Testini, PhD student
Charlotte Wikner, research engineer

Funding during 2015

Swedish Cancer Society, 2 000 kSEK
Swedish Research Council, 1 800 kSEK
World Wide Cancer Research, 600 kSEK

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New Strategies to Control Tumor Angiogenesis and Vascular Permeability

Elisabetta Dejana

Our research is focused at understanding the mechanisms that regulate the formation of the vascular system during embryo development and in tumours.
One of the aspects of anti-cancer therapies concerns the possibility of inhibiting tumour growth by blocking blood supply. The simple idea is that, if starved, the tumor will not grow but, on the contrary, will shrink and become more susceptible to chemotherapy and radiotherapy.

Cancer cells induce the formation of their own vascular system by recruiting new vessels from the host. However, the resulting vasculature is structurally and functionally abnormal. The vessels are leaky, tortuous, dilated and have lost hierarchy. The endothelial cells lining these vessels have aberrant morphology and are frequently retracted exposing the underlying matrix and tumour cells to the blood stream.

These structural abnormalities cause edema and hemorrhages contributing to interstitial hypertension, hypoxia, and acidosis. Impaired blood supply and interstitial hypertension create areas of necrosis and interfere with the homogeneous delivery of therapeutics.

These observations suggest that normalization of tumour vessels may be important to improve perfusion of the tumour microenvironment and, ultimately, improve cancer treatment. Furthermore, the normalized vasculature may be more resistant to tumour cell infiltration and metastatic dissemination.

Other pathologies are characterized by an abnormal and fragile vasculature. An example is Cerebral Cavernous Malformation (CCM), a genetic disease where the vessels form multiple lumen malformations in the brain vasculature, as described below in more detail.

Our research aims to clarify the mechanisms behind the vascular abnormalities detected in the tumor vasculature or in other pathologies. Our approach includes studies *in vivo*, using tumour models and genetically modified organisms, and *in vitro*, using cultured endothelial cells of different origin.

The role of adhesion proteins at cell-to cell junctions in angiogenesis and vascular stability

Abdallah Abu Taha

Endothelial cells form a continuous layer on the internal aspect of the vasculature that controls vascular permeability to inflammatory cells and plasma solutes. The integrity of the endothelium is sustained by cell-to-cell junctions and, in particular, adherens junctions (AJ). More specifically, AJ are formed by a transmembrane, endothelial specific, adhesive protein called VE-cadherin that promotes cell-to-cell adhesion and is linked inside the cells to cytoskeletal and signalling proteins. AJ besides their adhesive properties, restrict cell growth, prevent apoptosis and control the formation of new vessels. We identified a series of transmembrane and cytoplasmic partners of VE-cadherin able to transfer intracellular signals and modify the expression of endothelial genes. Several of these genes encode proteins that regulate endothelial cell growth and apoptosis and constitute potential targets for drug interventions geared towards modulation of vascular stability and angiogenesis.

Abdallah Abu Taha previously studied the dynamics of AJ assembly and disassembly in cultured endothelial cells. He developed a relatively large set of fluorescence tagged constructs including VE-cadherin and few intracellular partners that allow the study of the organization and disorganization of junctions in different functional conditions. Future work includes the development of genetically modified *in vivo* mouse models expressing

fluorescent VE-cadherin or other AJ components to investigate junctions' organization during vascular development in different experimental conditions.

Gene expression profile during vascular maturation at different postnatal stages and in pathology

Sara Cunha and Veronica Sundell

Early stages of vascular development include endothelial cell differentiation in a network of arteries, veins, and lymphatics. Subsequently, to respond to the specific needs of the organs, endothelial cells acquire specialized properties such as permeability control, expression of specific trans-cellular transport systems, membrane adhesive molecules, and others. Endothelial cell differentiation depends on communication between the surrounding tissues, that is mediated by growth and differentiation factors able to activate specific gene expression programs.
Vascular maturation and differentiation starts in the embryo but proceeds further after birth.

Strikingly, the inactivation of genes important in vascular development (such as growth factor receptors, VE-cadherin or other adhesion molecules) leads to major vascular problems in the embryo and in pups but may be almost ineffective in adult mice. We hypothesized therefore that vascular stability is regulated by specific genes upregulated during vascular maturation.

Sara Cunha who is an expert of *in vivo* models of inflammation and tumor angiogenesis, is studying endothelial gene expression at different stages of vascular development in pups using RNA seq analysis. The genes associated to immature or mature vascular conditions will be selected and further investigated. This first analysis will be further extended to the tumor vasculature and to CCM pathology.

Cerebral Cavernous Malformations (CCM)

Joppe Oldenburg

CCM is a genetic, familial and sporadic, disease characterized by vascular malformations concentrated in the central nervous system, typically formed by multiple lumens and particularly prone to bleeding. This pathology may result in several neurological symptoms, including headaches, seizures, paralyses and hemorrhagic stroke. CCM is the most frequent cause of hemorrhagic stroke in infancy. To date the only therapy available is surgery, however, surgery is frequently hazardous depending on the location of the vascular malformation.

In humans, loss of function mutations in any one of three independent genes known as CCM1 (Krit 1), CCM2 (MGC4607) and CCM3 (PDCD10) have been linked to the development of CCM. The vascular phenotype is largely superimposable in patients missing any one of the three genes.

Similarly to patients, in murine models, the vascular phenotype can be reproduced by endothelium-specific loss-of-function mutations of any one of these three CCM genes suggesting that they act in concert. Although CCM genes are expressed in the endothelium of different types of vessels the vascular malformations are present predominantly, if not only, in the brain microcirculation.

In our research work we observed that the endothelial cells lining the vascular CCM malformations change their morphological and functional characteristics, acquiring mesenchymal markers and elongated morphology. We found that two major signaling pathways (Wnt and TGF beta) are responsible for these changes of endothelial cells. Inhibitors of these pathways were found to prevent the endothelial to mesenchymal switch and the development of CCM malformations.

Joppe Oldenburg studied the role of endothelial cell-to-cell junctions in the control of permeability and vascular stability. He is now engaged to identify available drugs able to inhibit the development of CCM malformations or to induce their regression.

Taking advantage of the *in vitro* models of the disease present in the lab he is now working on the optimization of a standardized assay to be used for a large screening of chemicals and/or known drugs. With the collaboration of the SciLife Lab at Stockholm this experience will be applied to robotic microplate based cellular assays in a validated environment. The SciLife Organization has access to libraries of several hundreds thousands small molecules and 94/384-well plate capable robots. Sci Life Lab is dedicated to drug discovery and development and may offer an invaluable opportunity for translating drug discovery ideas into medical therapies.

Group members during 2015

Elisabetta Dejana, professor, group leader
Abdallah Abu Taha, post doc
Sara Cunha, post doc
Joppe Oldenburg, post doc
Veronica Sundell, technician

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Publications 2013-2015

(The group came to IGP in 2015 and most papers have therefore not been published with IGP as affiliation)

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Tumor Vascular Biology

Anna Dimberg

Blood vessel formation and inflammation are closely linked processes that affect the clinical outcome of several pathological conditions, including cancer. Endothelial cells, lining the inside of vessels, are central players in both these processes. They initiate the formation of new vessels after growth factor stimulation and regulate extravasation of inflammatory cells from the blood stream into the tissue.

Tumor vessels are morphologically and functionally distinct from normal vessels, at least partially as a consequence of ongoing angiogenesis and extensive growth factor stimulation. Proteins specifically expressed in endothelial cells during tumor angiogenesis may constitute new targets for cancer treatment. Importantly, heterogeneous protein expression in tumor endothelium may affect leukocyte recruitment, permeability and establishment of a vascular niche. The focus of our research is to understand how the vasculature affects cancer progression through regulation of the tumor microenvironment.

Molecular regulation of vascular abnormalization in glioblastoma

Lei Zhang, Roberta Langenkamp, Kalyani Vemuri, Hua Huang, Maria Georganaki, Liisi Laaniste, and Anna Dimberg

Glioblastoma, the most aggressive type of glioma, are characterized by high mitotic activity, nuclear atypia, microvascular proliferation, hemorrhage and necrosis. The median survival of adult glioblastoma patients is only twelve months. Extensive angiogenesis and markedly abnormal vessels are a hallmark of glioblastoma, leading to enhanced permeability and brain oedema. However, the molecular mechanisms that underlie the extensive morphological and functional changes observed in glioblastoma vasculature are largely unknown.

To have previoiusly identified 95 genes that are differentially expressed in glioblastoma vessels and found that many of these genes are induced in response to growth factors highly expressed in the tumor microenvironment. Among these genes, we have demonstrated that CD93 regulates the endothelial cytoskeleton and is important for formation of functional tumor vessels in glioblastoma. We are also investigating other proteins highly expressed in glioblastoma vessels to determine how these contribute to aberrant vascular function and tumor progression in glioblastoma.

Pleiotrophin is a small heparin-binding growth factor that is frequently expressed in human glioblastoma and low-grade glioma, but not detectable in normal adult brain tissue. It is considered to be a pro-angiogenic growth factor, but its net effect appears to be context dependent as it can also oppose angiogenesis in some systems. In glioma, pleiotrophin has been shown to affect migration and proliferation of tumor cells that express its receptors. Our results show that pleiotroiphin is a key inducer of vascular abnormalization in glioblastoma. We are currently exploring different possibilities to target pleiotrophin and thereby normalize tumor vessels in glioblastoma.

Cross-talk between pro-angiogenic and pro-inflammatory signalling pathways in the tumor microenvironment and its impact on immunotherapy

Hua Huang, Maria Georganaki, Luuk van Hooren and Anna Dimberg

Tumor growth is significantly affected by recruitment of inflammatory cells. This process is regulated by *endothelial activation*, endothelial up-regulation of adhesion molecules that capture leukocytes and enable slow rolling, firm adhesion and transmigration into the tissue. Pro-angiogenic signalling in the tumor microenvironment affects endothelial activation

through negative crosstalk with pro-inflammatory signalling pathways. Also, the aberrant architecture and blood flow in combination with changes in endothelial gene expression may limit effector lymphocyte recruitment into the tumor.

The success of cancer immunotherapy relies on efficient recruitment of immune cells into the tumor mass. Despite recent breakthroughs, the tumor vasculature still presents a hurdle for infiltrating leukocytes that limits the efficacy of cancer immunotherapy in solid tumors. We have shown that inhibition of VEGFR-signaling will lead to tumor vessel up-regulation of chemokines necessary recruitment of T-cells. Future efforts include investigating possible benefits and pitfalls of combining vascular targeting with immunotherapy approaches. The goal is to find new combinatorial therapies for cancer.

Group members during 2015

Anna Dimberg, researcher, group leader
Maria Georganaki, PhD student
Hua Huang, PhD student/ assistant undergoing research training
Liisi Laaniste, degree project student
Elise Langenkamp, post doc
Roberta Lugano, post doc
Luuk van Hooren, PhD student
Kalyani Vemuri, degree project student
Lei Zhang, PhD student/assistant undergoing research training

Dissertations during 2015

Hua Huang, Endothelial activation and inflammation in the tumor microenvironment, May 8, 2015.

Lei Zhang, Molecular Regulation of Vascular Abnormalization and Its Role in Glioma. May 30, 2015

Funding during 2015

Swedish Cancer Society, 600 kEK
Swedish Research Council, 1 000 kSEK
Swedish Childhood Cancer Foundation, 300 kSEK
Swedish Childhood Cancer Foundation, NBCNS 400 kSEK
Emil och Vera Cornelius stiftelse, 220 kSEK
EU, FP7 MC ITN (TIMCC) 1 501 kSEK

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Regulation of Lymphatic Vasculature

Taija Mäkinen

Lymphatic vasculature constitutes a network of vessels critical for the maintenance of the body's fluid balance. Failure of the lymphatic vessels can lead to a disabling disease called lymphoedema for which there is no cure or effective treatment. Recent studies have revealed important new roles of lymphatic vasculature in inflammation, immunity, lipid metabolism, blood pressure regulation and cancer metastasis. Understanding mechanisms of lymphangiogenesis may thus enable development of new therapies for common diseases that affect a large number of people worldwide.

Our laboratory aims to understand, at the molecular level but in the context of a living organism, the regulation of lymphatic vascular morphogenesis. We utilise and develop advanced mouse genetic tools to spatially and temporally control expression of genes in specific cell types of interest. By identifying and functionally characterising genes causative of hereditary lymphoedema we additionally aim to uncover mechanisms of vascular development that are directly relevant to human pathology.

Organ- and vessel-type –specific mechanisms of lymphatic development

Ines Martinez-Corral, Maria Ulvmar, Yan Zhang, Yang Zhang, Henrik Ortsäter

The lymphatic system is composed of a hierarchy of vessels with specific features serving their unique functions: the blind-ended lymphatic capillaries that absorb the interstitial fluid and the collecting lymphatic vessels that transport the lymph to the cardiovascular system. Failure of the lymphatic vessels, caused by a genetic defect (primary) or damage following surgery or radiation therapy (secondary) can lead to lymphoedema, which is a progressive and lifelong condition characterised by gross swelling of the affected tissue. Notably, several primary lymphoedemas are characterised by defects that affect specifically either the collecting vessels or the capillaries. In addition, specific area(s) of the body are affected in different types of lymphoedemas. What underlies tissue-specific vessel failure is not understood yet this knowledge is instrumental in designing therapeutic strategies for lymphoedema and other lymphatic disorders that are currently lacking. In this project we will identify genes and mechanisms required for organ-specific lymphatic vascular development by characterising the features of specific lymphatic vascular beds, and by identifying and functionally characterising genes regulating lymphatic development in an organ- and/or vessel-type specific manner using genetic mouse models.

Functional characterisation of causative genes for human primary lymphoedema

Ines Martinez-Corral, Andrea Taddei (London), Maike Frye

Recently gained insights into mechanisms of lymphangiogenesis have been driven by the characterisation of animal models with specific lymphatic defects, and identification of genes causative of human primary lymphoedemas. In collaboration with Pia Ostergaard, Steve Jeffery, Peter Mortimer and their teams at St George's Hospital in London, we have recently identified *GATA2* and *KIF11* as two novel causative genes for primary lymphoedema by whole-exome sequencing. We are currently investigating the biological function of *GATA2* and *KIF11* in lymphatic development by combining state-of-the-art mouse genetics with in vitro studies on primary lymphatic endothelial cells. The results from this project are expected to increase our understanding of normal lymphatic development and pathophysiological mechanisms involved in lymphoedema and other lymphatic disorders.

Group members during 2015

Taija Mäkinen, senior lecturer, group leader
Maike Frye, post doc
Ines Martinez-Corral, researcher
Henrik Ortsäter, research engineer
Maria Ulvmar, researcher
Andrea Taddei (post doc, Cancer Research UK London Research Institute)
Yan Zhang, post doc
Yang Zhang, PhD student
Sofie Wagenius, laboratory assistant

Funding during 2015

European Research Council (consolidator grant), 370 kSEK
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Publications 2013-2015

(The group came to IGP in 2013 and some papers have therefore not been published with IGP as affiliation)

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